08-18-00

JC853 U.S. PTO
08/17/00

PATENT
Docket No. 600-41-PA (17377)

"EXPRESS MAIL" MAILING LABEL NO.: EL625975676US
DATE OF DEPOSIT August 17, 2000
I HEREBY CERTIFY THAT THIS PAPER OR FEE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE "EXPRESS MAIL POST OFFICE TO ADDRESSEE" SERVICE UNDER 37 CFR 1.10 ON THE DATE INDICATED ABOVE AND IS ADDRESSED TO:

Assistant Commissioner of Patents
Washington, D. C. 20231

Renne M. Fischer
Typed Name of Person Mailing Document

Signature

09/640852 U.S. PTO
08/17/00

TO THE COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D. C. 20231

NEW APPLICATION TRANSMITTAL

Dear Sir:

Transmitted herewith for filing is the patent/design patent application of:

Inventor(s): Alissar NEHME; Richard L. BEARD; and Roshantha A. CHANDRARATNA

For: TREATMENT OF TUMORS WITH ACETYLENES DISUBSTITUTED WITH A PHENYL OR HETEROAROMATIC GROUP AND A SUBSTITUTED CHROMANYL, THIOCHROMANYL OR TETRAHYDROQUINOLINYL GROUP IN COMBINATION WITH OTHER ANTI-TUMOR AGENTS

Enclosed are the following:

18 pages of Specification; 6 pages of Claims; 1 pages Abstract;

16 sheet(s) of Formal/Informal Drawings;

4 pages Executed/Unexecuted Declaration/Power of Attorney;

[XXX] An Assignment of the Invention to: Allergan Sales, Inc.

[ ] Verified Statement(s) to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27 for: _____;

[ ] Information Disclosure Statement of _____ pages, including Form PTO-1449 of _____ pages and copies of _____ references;

[XX] (other) Assignment Recordation Cover Sheet for Patents

The Filing Fee has been calculated as shown below:

FOR:	(Col. 1) # FILED	(Col. 2) # EXTRA	SMALL ENTITY RATE	SMALL ENTITY BASIC FEE	OTHER THAN A SMALL ENTITY RATE	OTHER THAN A SMALL ENTITY BASIC FEE
BASIC FEE				$ 345		$ 690
TOTAL CLAIMS	35 -20=	15	$ 9	$	$ 18	$ 270
INDEP. CLAIMS	2 -3=	0	$39	$	$ 78	$ -0-
MULTIPLE DEPENDENT CLAIM PRESENTED			$130	$	$260	$ 260
*If the difference in Col. 1 is less than zero, enter "0" in Col. 2.			TOTAL	$	TOTAL	$1,220

[ ] A check in the amount of $_____ to cover the Filing Fee is enclosed.

[ ] Check includes $40.00 for the Assignment Recording Fee.

[XX] **PLEASE CHARGE/DEPOSIT ACCOUNT NO. 01-0885 FOR ALLERGAN/ALL FEES INCLUDING ANY DEFICIENCIES IN THE ABOVE-REFERENCED FEE CALCULATIONS**, Including the Assignment Recordation Fees.

By: RJ Baran
Robert J. Baran, Reg. No. 25,806
Authorized Signature for ALLERGAN, INC.
Deposit Account No. 01-0885

By: Gabor L. Szekeres
Gabor L. Szekeres Reg.#: 28,675
KLEIN & SZEKERES, LLP
4199 Campus Drive, Suite 700
Irvine, California 92612
Phone (949) 854-5502
FAX (949) 854-4897

TREATMENT OF TUMORS WITH ACETYLENES DISUBSTITUTED WITH A PHENYL OR HETEROAROMATIC GROUP AND A SUBSTITUTED CHROMANYL, THIOCHROMANYL OR TETRAHYDROQUINOLINYL GROUP IN COMBINATION WITH OTHER ANTI-TUMOR AGENTS

BACKGROUND OF THE INVENTION

1. Field of the Invention

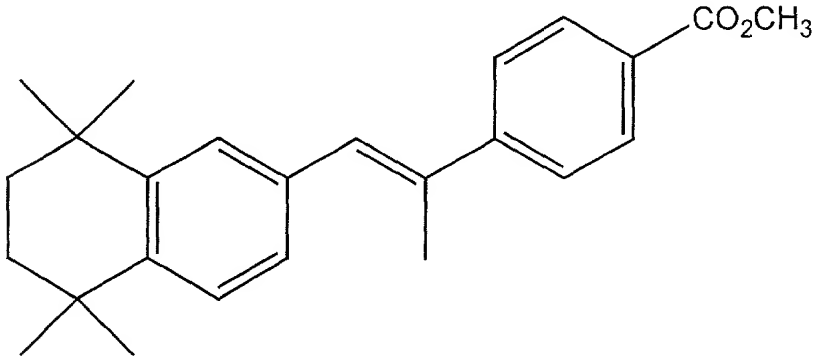
The present invention relates to the use of acetylenes disubstituted with a phenyl or heteroaromatic group and a substituted chromanyl, thiochromanyl or tetrahydroquinolinyl group for the treatment of tumors in combination with other anti-tumor agents. More particularly the present invention relates to the use of ethyl 6-[2-(4,4-dimethylthiochroman-6-yl)ethynyl]nicotinate for the treatment of malignancies, particularly carcinoma of the breast and human myeloid leukemia, in combination with interferons and other anti-tumor agents.

2. Background Art

Naturally occurring retinoic acid and related compounds, generally called retinoids, have been known in the pharmaceutical, medical and related arts to have of important biological activity, including prevention and inhibition of malignant cell proliferation. A vast volume of patent and scientific literature exists describing the synthesis of retinoid compounds, their biological activities and investigations aimed at discovering the varying modes of action of retinoids in human and other biological systems, *in vitro* and *in vivo* as well.

Specifically, it is generally accepted in the art that in the anti-cell-proliferative or anti-tumor field, pharmaceutical compositions having a retinoid-like compound or compounds as the active ingredient are useful for treating or preventing hyperproliferative disorders of the skin, and other premalignant and malignant hyperproliferative diseases such as cancers of the

breast, skin, prostate, cervix, uterus, colon, bladder, esophagus, stomach, lung, larynx, oral cavity, blood and lymphatic system, metaplasias, dysplasias, neoplasias, leukoplakias and papillomas of the mucous membranes and in the treatment of Kaposi's sarcoma. Still more specifically, there are published reports in the art that certain retinoid compounds act additively and some even synergistically with other known anti-tumor chemotherapeutic agents, such as interferons and other drugs, in several carcinoma of the breast cell cultures to suppress or inhibit the proliferation of the cancer cells. The publication by *Fanjul et al.* in Cancer Research **56**, 1571 - 1577 (1996) describes assays of several retinoid compounds, including a compound designated in the publication as SRI 11220 in combination with interferon in several carcinoma cell lines, and states that in some of the cell lines the anti-proliferative activity of the compound SRI 11220 and interferon was synergistic. The structure of this prior art compound SRI 11220 is shown below.



SRI 11220 (Prior Art)

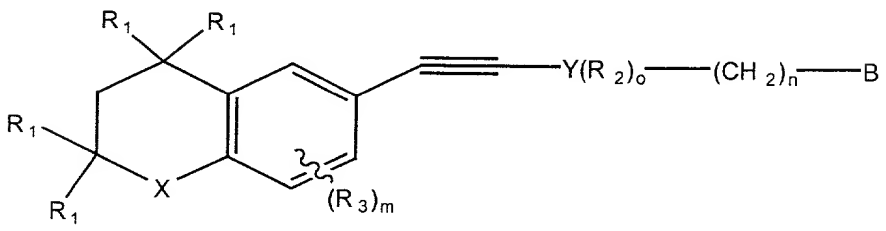
A publication by *Toma et al.* in International Journal of Oncology **10:** 597 - 607 (1997) describes synergistic effects of certain other retinoids, such as all trans retinoic acid (tRA) with α interferon (α IFN) and synergistic effect with other chemotherapeutic agents such as tamoxifen (TAM) in MCF-7 human breast cancer lines. As further background to the present invention it

is noted that a publication by *Kurbacher et al.* in Cancer Letters **103** (1996) 183 - 189 describes synergistic action of vitamin C with certain chemotherapeutic anti-tumor agents in MCF-7 and MDA-MB 231 human carcinoma cell lines.

United States Patent Nos 4,810,804, 4,980,369, 5,045,551, and 5,089,509 describe acetylenes disubstituted with a phenyl or heteroaromatic group and a substituted chromanyl, thiochromanyl or tetrahydroquinolinyl group having retinoid like activity. United States Patent Nos. 5,602,130 and 6,090,826 disclose a method of treating diseases or conditions susceptible to treatment by retinoids, with acetylenes disubstituted with a heteroaromatic group and a substituted chromanyl, thiochromanyl or tetrahydroquinolinyl group. United States Patent No. 5,089,509 is of particular relevance as background to the present invention, because it discloses the synthesis of ethyl 6-[2-(4,4-dimethylthiochroman-6-yl)ethynyl]nicotinate which is the preferred compound used in the method of treatment of the present invention. Ethyl 6-[2-(4,4-dimethylthiochroman-6-yl)ethynyl]nicotinate is also known by its trade name TAZAROTENE®, and is often referred to in the present specification (including the drawing figures) simply as "tazarotene".

SUMMARY OF THE INVENTION

The present invention relates to the use of the compounds of **Formula 1**



FORMULA 1

where $\mathbf{R_1}$ is independently H or lower alkyl of 1 to 6 carbons;

$\mathbf{R_2}$ and $\mathbf{R_3}$ are independently H, lower alkyl of 1 to 6 carbons, F, Cl, Br, I, alkoxy of 1 to 6 carbons, or fluoroalkoxy of 1 to 6 carbons;

m is an integer 0 to 3;

o is an integer 0 to 4;

n is 0-5;

Y is phenyl, naphthyl, or a heteroaryl group selected from a group consisting of pyridyl, thienyl, furyl, pyridazinyl, pyrimidinyl, pyrazinyl; oxazolyl, thiazolyl, or imidazolyl, and

B is COOH, a pharmaceutically acceptable salt thereof, CON$\mathbf{R_6R_7}$ or COO$\mathbf{R_8}$ where $\mathbf{R_6}$ and $\mathbf{R_7}$ independently are hydrogen or an alkyl group of 1 to 6 carbons and $\mathbf{R_8}$ is alkyl of 1 to 6 carbons,

for the treatment of a malignant tumor or condition in a mammal in need of such treatment, in combination with one or more other anti-tumor agent, preferably in combination with an interferon.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing synergism in the anti-proliferative effects of a combination of the compound tazarotene (**Formula 3**) and of α interferon (IFN-a or IFNα) in SK-BR-3 cells.

Figure 2 is a graph showing the anti-proliferative effects of a combination of the compound tazarotene (**Formula 3**) and of α interferon (IFN-a or IFNα) in T-47D cells.

Figure 3 is a graph showing synergism in the anti-proliferative effects of a combination of the compound tazarotene (**Formula 3**) and of β interferon (IFN-b or IFNβ) in SK-BR-3 cells.

Figure 4 is a graph showing synergism in the anti-proliferative effects of a combination of the compound tazarotene (**Formula 3**) and of β interferon (IFN-b or IFNβ) in T-47D cells.

Figure 5 is a graph showing synergism in the anti-proliferative effects of a combination of the compound tazarotene (**Formula 3**) and of γ interferon (IFN-g or IFNγ) in SK-BR-3 cells.

Figure 6 is a graph showing the anti-proliferative effects of a combination of the compound tazarotene (**Formula 3**) and of γ interferon (IFN-g or IFNγ) in T-47D cells.

Figure 7 is another graph showing synergism in the anti-proliferative effects of a combination of the compound tazarotene (**Formula 3**) and of α interferon (IFN-a or IFNα) in SK-BR-3 cells.

Figure 8 is another graph showing the anti-proliferative effects of a combination of the compound tazarotene (**Formula 3**) and of α interferon (IFN-a or IFNα) in T-47D cells.

Figure 9 is another graph showing synergism in the anti-proliferative effects of a combination of the compound tazarotene (**Formula 3**) and of β interferon (IFN-b or IFNβ) in SK-BR-3 cells.

Figure 10 is another graph showing synergism in the anti-proliferative

effects of a combination of the compound tazarotene (**Formula 3**) and of β interferon (IFN-b or IFNβ) in T-47D cells.

Figure 11 is another graph showing synergism in the anti-proliferative effects of a combination of the compound tazarotene (**Formula 3**) and of γ interferon (IFN-g or IFNγ) in SK-BR-3 cells.

Figure 12 is another graph showing the anti-proliferative effects of a combination of the compound tazarotene (**Formula 3**) and of γ interferon (IFN-g or IFNγ) in T-47D cells.

Figure 13 is a graph showing synergism in the anti-proliferative effects of a combination of the compound tazarotene (**Formula 3**) and of α interferon (IFN-alpha or IFNα) in HL-60 cells.

Figure 14 is a graph showing synergism in the anti-proliferative effects of a combination of the compound tazarotene (**Formula 3**) and of β interferon (IFN-beta or IFNβ) in HL-60 cells.

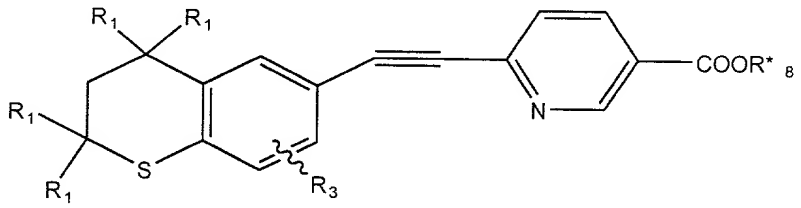
Figure 15 is another graph showing synergism in the anti-proliferative effects of a combination of the compound tazarotene (**Formula 3**) and of α interferon (IFN-alpha or IFNα) in HL-60 cells.

Figure 16 is another graph showing synergism in the anti-proliferative effects of a combination of the compound tazarotene (**Formula 3**) and of β interferon (IFN-beta or IFNβ) in HL-60 cells.

COMPOUNDS USED IN THE METHODS OF TREATMENT OF THE INVENTION

The general formula of the compounds used in the methods of treatment of the invention is shown in **Formula 1**. Among the compounds shown in that formula, the use of those are preferred where the variable **Y** designates pyridine. Even more preferred are those where the pyridine moiety is 2,5 substituted. (Substitution in the 2,5 positions in the "pyridine" nomenclature corresponds to substitution in the 6-position in the "nicotinic acid" nomenclature.) As far as the $(CH_2)_n$ group is concerned, compounds are preferred where **n** is 0. Preferably **B** is COOH or $COOR_8$ where R_8 is lower alkyl of 1 to 3 carbons. R_1 preferably designates H or methyl, and R_2 and R_3 are pereferably H or lower alkyl. The variable **X** preferably represents S or O, still more preferably S.

A more preferred group of compounds utilized in the methods of the invention is depicted by **Formula 2**

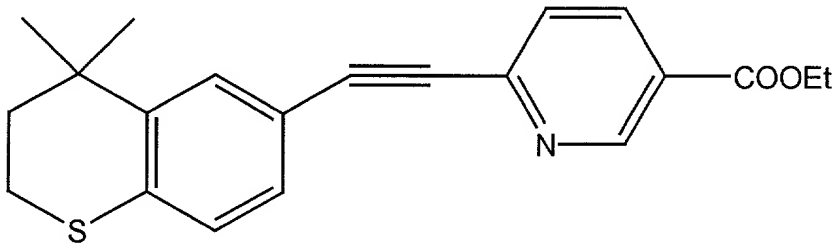


FORMULA 2

where R_1 is H or methyl, R_3 is H or methyl, and R^*_8 is H, or lower alkyl of 1 to 3 carbons, or a pharmaceutically acceptable salt of said compound. The compounds of **Formula 1** and of **Formula 2** can be obtained in accordance with the synthetic procedures described in United States Patent Nos. 4,810,804, 4,980,369, 5,045,551, and 5,089,509, each of which is expressly incorporated herein by reference.

The presently most preferred compound used in the methods of

treatment of the present invention is ethyl 6-[2-(4,4-dimethylthiochroman-6-yl)ethynyl]nicotinate (tazarotene) the structure of which is disclosed by **Formula 3**. Tazarotene is described as example 6 in the specification of United States Patent No. 5,089,509.



FORMULA 3 (tazarotene)

It should be understood in connection with the description of the compounds used in the methods of treatment of the present invention that a pharmaceutically acceptable salt is any salt which retains the activity of the parent compound and does not impart any deleterious or untoward effect on the subject to which it is administered and in the context in which it is administered. Pharmaceutically acceptable salts may be derived from organic or inorganic bases. The salt may be a mono or polyvalent ion. Of particular interest are the inorganic ions, sodium, potassium, calcium, and magnesium. Organic salts may be made with amines, particularly ammonium salts such as mono-, di- and trialkyl amines or ethanol amines. Salts may also be formed with caffeine, tromethamine and similar molecules.

It should be further understood in connection with the description of the compounds used in the methods of treatment of the present invention that in **Formulas 1** and **2**, the substituents $\mathbf{R_2}$ and $\mathbf{R_3}$ are optional, meaning that when the variables **m** and **o** have the value of 0 (zero), then the respective ring is hydrogen substituted; in other words the ring bears no $\mathbf{R_2}$ or $\mathbf{R_3}$ substituent other than hydrogen.

ANTI-PROLIFERATIVE EFFECTS OF THE COMPOUNDS UTILIZED IN THE METHODS OF TREATMENT OF THE INVENTION

The anti-proliferative effects of the compounds used in accordance with the invention are demonstrated by assay procedures well accepted in the art. These assays are performed on the preferred compound, tazarotene (the compound of **Formula 3**) without and also in combination with human recombinant α, β and γ interferon which are anti-tumor agents well known in the art. The materials and the assays procedures are described in detail below.

The SK-BR-3, T-47D and HL-60 cell cultures in which the assay procedures were performed are also well known and are available from sources well known in the art. Specifically, as is known, T-47D is an estrogen receptor positive (ER^+) human breast cancer cell line, and SK-BR-3 is an estrogen receptor negative (ER^-) human breast cancer cell line. HL-60 is a well known human myeloid leukemia cell line. The assay procedure for the breast cancer lines itself is well known in the art and involves determining incorporation of 5-bromo-2'-deoxyuridine (BrdU) into the cells. As is known, incorporation of less BrdU represents less cell proliferation (inhibition of cell proliferation), and this assay is accepted in the art as a measure of anti-proliferative or anti-tumor activity of the assayed agent or agents. The assay procedure for the HL-60 cell line is also well-known in the art. It involves measuring the concentration of formazan dye which is cleaved from 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide by viable HL-60 cells.

When a combination of two or more anti-proliferative or potentially anti-proliferative agents is assayed, the results may indicate less inhibition of proliferation than what we would be expected if the effects of the individual agents were additive, or the effects may represent the mathematical product of the expected effects of the two agents (additive inhibition). Alternatively, the

inhibition actually observed experimentally may be greater than what would be expected as a simple product of the effects of the two agents. Such synergistic anti-tumor or antiproliferative effect is highly desirable, and as is described below was observed in several assays when tazarotene (**Formula 3**) was used in combination with human recombinant interferon. This synergistic effect of the compounds used in the invention with interferon in the treatment of malignancies, and especially in treatment of breast cancer and of acute human myeloid leukemia is not expected based on the prior art and is unobvious and surprising. The materials and procedures of the assays as well as the mathematical criteria for determining synergistic effects are described below.

Materials, Assay Methods and Criteria for Determining Synergism

Reagents

The human recombinant interferon-alpha (IFN-α) and human recombinant interferon-beta (IFN-β) were purchased from Sigma Chemicals Co. (St Louis, MO). Human recombinant interferon-gamma (IFN-γ) was purchased from Roche Diagnostics (Indianapolis, IN). The stock solutions were stored at −70, 4, and −20 °C for IFN-α, IFN-β and IFN-γ, respectively. IFN working solutions were prepared before use by dilutions in the culture medium. 5 mM stock solution for tazarotene (**Formula 3**) was prepared in DMSO, which was subsequently diluted in culture medium to the indicated final concentration.

Culture of Breast Cancer Cell Lines

The estrogen receptor-positive (ER^+) cell line T-47D and the ER^- cell line SK-BR-3 were cultured in Dulbecco's modification of Eagle's medium (DMEM Gibco BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 2 mM L-glutamine and 1% antibiotics-antimycotics (Gibco BRL). Cell lines were obtained from the American Type

Culture Collection (ATCC, Rockville, MD, HTB-133 and HTB-30 for T-47D and SK-BR-3, respectively). Cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2.

Culture of HL-60 Acute Myeloid Leukemia Cells

The human myeloid leukemia cell line HL-60 was cultured in Iscove's modified Dulbecco's medium (IMDM Gibco BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 2 mM L-glutamine and 1% antibiotics-antimycotics (Gibco BRL). HL-60 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, CCL-240). Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2

Cell Proliferation Assay in Breast Cancer Cell Lines

Proliferation of cancer cell lines was determined using a commercial cell proliferation kit (Roche Diagnostics), essentially following the instructions of the manufacturer. Cells were seeded into 96-well tissue culture plates (Corning Incorporated, Corning, NY) at a concentration of 3000 cells/well. After 24 hours, cells were treated continuously with tazarotene (**Formula 3**) and/or interferons (IFNs) or solvent alone. The appropriate concentrations of tazarotene (**Formula 3**) used in this study were between 10^{-11}M and 10^{-6}M; IFN concentrations were between 10 and 1000 Unit/ml. Culture media were changed every 72 hours. After 7days, 10 µl of 5-bromo-2'-deoxyuridine (BrdU) was added to each well. Incubation with BrdU was stopped 24 hours later by adding 100 µl of anti-BrdU antibody to each well. The amount of BrdU incorporated into the DNA of proliferating cells was assessed by measuring absorbance at 450 nm. Each experiment was performed in triplicate in three independent experiments.

Cell Proliferation Assay (MTT) in HL-60 Leukemia Cell Line

Proliferation of the HL-60 leukemia cell line was determined by a cell

viability and non-radioactive commercial cell proliferation kit (MTT assay ; Roche Diagnostics, Indianapolis, IN), essentially by following the instructions of the manufacturer. Cells were seeded into 96-well tissue culture plates (Corning Incorporated, Corning, NY) at a concentration of 1000 cells/well. After 24 hours, the cells were treated continuously with tazarotene (**Formula 3**) and/or IFNs or solvent alone. The appropriate concentrations of tazarotene (**Formula 3**) used in this study were between 10^{-11}M and 10^{-6}M ; IFN concentrations were between 0.1 and 1000 Unit/ml. Culture media were changed every 72 hours. After 6 days, 10 µl of MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) was added to each well. The reaction was stopped after 4 hours of incubation by adding 100 µl of 10% SDS in 0.01 M HCl. The quantification of viable cells, capable of cleaving MTT to form a formazan dye, was assessed by measuring absorbance at 590 nm. All determinations were performed in triplicate in three independent experiments.

Criteria for Synergism

The growth inhibition observed for a combined treatment with tazarotene (**Formula 3**) and IFNs was analyzed for both synergistic and additive effects. The criteria for these effects have been discussed by three different groups (*Aapro et al.,* Cancer Chemother. Pharmacol., 10: 161-166, 1983, *Marth et al.,* J. Natl. Cancer Inst., 77:1197-1202, 1986, *Kurbacher et al.,* Cancer Letters, 103: 183-189, 1996). The mathematical multiplication of the two surviving fractions after the treatment of either with tazarotene (**Formula 3**) or with the respective interferon is the calculated value for simple additivity of both agents in combination. This calculated value is compared to the actual value observed to determine the nature of the combination effect. Statistical significance of synergistic effects is determined by using the two-sided Student's t-Test. Synergism or inhibition was determined for each experiment individually, with the *P* value being 0.05 in comparison to the

simple additivity hypothesis. **Table 1** below shows the mathematical expressions for the criteria of two agents being synergistic, additive, subadditive and antagonistic, respectively.

Table 1. Definitions of drug combination effects[a]

Synergistic	$SF_{A+B} < (SF_A) \times (SF_B)$
Additive	$SF_{A+B} = (SF_A) \times (SF_B)$
Subadditive	$SF_{A+B} > (SF_A) \times (SF_B)$ And $< SF_B$ when $SF_A > SF_B$
Antagonistic	$SF_{A+B} > (SF_A) \times (SF_B)$

[a] SF_A: Surviving fraction from treatment A; SF_B: Surviving fraction from treatment B;
SF_{A+B}: Surviving fraction from treatment A plus B.

Anti-Proliferative Effects Determined by the Assays

Referring now to the graphs of **Figures 1** through **16**, each of these represents the results obtained in the above described assays where SK-BR-3, T-47D and HL-60 cells, respectively, were treated with a combination of tazarotene (**Formula 3**) and human recombinant interferon (IFN) α, β, and γ, respectively. In the graphs of **Figures 1- 12**, pertaining to assays with SK-BR-3 cells and T-47D cells, the incorporation of 5-bromo-2'-deoxyuridine (BrdU) is plotted on the Y (vertical) axis and varying concentration of tazarotene (**Formula 3**) or varying concentration of IFNα. IFNβ or of IFNγ, respectively, is plotted on the X (horizontal) axis. The concentration of the interferons is expressed in international units, as is accepted in the art, whereas the molar concentration of tazarotene (**Formula 3**) is plotted on a logarithmic scale. Each graph includes a curve indicating results with one agent only, actual experimental results with the combination of the two agents (tazarotene, and the respective interferon), and a theoretical curve which is calculated in the manner described above, assuming for the

calculation that the effects of the two agents would be simply additive. The incorporation of BrdU is plotted on a percentage basis relative to the situation when the agent of varying concentration in the respective graph was not used (0 concentration represents 100 % incorporation).

The graphs of **Figures 13 - 16** are analogous, except that in these graphs the quantity of viable cells capable of cleaving MTT to form formazan dye, as measured by the quantity of fomazan dye (itself measured by absorbance at 590 nm) is plotted on the vertical (Y) axis.

Referring now specifically to the graph if **Figure 1**, in the assay of SK-BR-3 cells depicted in that graph the concentration of IFNα was 100 International Units (U) per ml, and the concentration of tazarotene was varied. It can be seen on the graph that the experimentally or actually observed inhibition of cell proliferation was significantly greater (less BrdU incorporation) than with IFNα alone, and significantly greater than the theoretically additive curve, thus showing a synergistic effect of tazarotene (**Formula 3**) and IFNα.. The graphs of **Figures 3** and **5**, similarly depict the results of assays in SK-BR-3 cells where the concentration of IFNβ or IFNγ was kept constant at 10U/ml and at 100U/ml respectively, and the concentration of tazarotene (**Formula 3**) was varied. The graphs of **Figures 3** and **5** also show significant synergistic effect of the combination treatment.

The graphs of **Figures 7**, **9**, and **11** again disclose the results of assays with SK-BR-3 cells. In these assays the concentration of tazarotene (**Formula 3**) was kept constant at 10 nM, and the concentration of IFNα, IFNβ or IFNγ was varied between 0 to 1000 International Units (0 to 1000 U) per milliliter (ml). These graphs reveal striking synergism.

The graphs of **Figures 2**, **4** and **6** disclose the results of assays with T-47D cells, where in analogy to the assays shown in graphs of **Figures 1**, **3** and **5** the concentration of tazarotene (**Formula 3**) was varied, and the

concentration of IFNα, IFNβ or IFNγ was kept constant at 100 U/ml. The graphs of these figures also shows synergism, although not as striking as in the assays with SK-BR-3 cells.

The graphs of **Figures 8**, **10** and **12** also disclose the results of assays with T-47D cells. In these assays, in analogy to the assays shown in graphs of **Figures 7**, **9** and **11**, the concentration of tazarotene (**Formula 3**) was kept constant at 10 nM, and the concentration of IFNα, IFNβ or IFNγ was varied between 0 to 1000 International Units (0 to 1000 U) per milliliter (ml). The graph of **Figure 8** (IFNα) reveals weak synergism, and the graph of **Figure 10** (IFNβ) shows significant synergism.

Figures 13 - 16 pertain to assays with HL-60 acute myeloid leukemia cells. In the assays disclosed by **Figures 13** and **14**, the concentration of IFNα or IFNβ was kept consntant at 100 U/ml , and the concentration of tazarotene (**Formula 3**) was varied. In the assays disclosed by the graphs of **Figures 15** and **16** the concentration of tazarotene (**Formula 3**) was kept constant at 50 nM, and the concetration of IFNα or IFNβ, respectively, was varied between 0 to 1000 U/ml. In these assays also, significant synergism was observed.

The foregoing results and particularly the synergism in the anti-proliferative effects on the two solid tumor cancer cell lines and in the HL-60 leukemia cells of tazarotene (**Formula 3**) and of human recombinant interferon is unexpected, surprising, and an indication that the compounds of **Formula 1** are useful for the treatment of diseases involving malignant cell-proliferation, such as solid tumors, particularly carcinoma of the breast, and leukemias, particularly acute myeloid leukemia. In fact, the foregoing assays indicated that the compounds of **Formula 1** are useful in combination therapy with interferon in breast cancer cell lines which are estrogen receptor positive (T-47D) and also in human breast cancer cell lines which are estrogen receptor negative (SK-BR-3).

Methods of Treatment, Modes of Administration

The compounds of **Formula 1** may be administered systemically or topically, depending on such considerations as the condition to be treated, need for site-specific treatment, quantity of drug to be administered, and numerous other considerations. For the treatment of breast cancer and many other forms of solid tumors, as well as in treatment of leukemias, the compounds of **Formula 1** are more likely to be administered systemically, in a pharmaceutical composition containing such excipients or inert components which are well known in the art pertaining to chemotherapy of tumors. More specifically, if a compound of **Formula 1** is to be administered systemically, it may be confected as a powder, pill, tablet or the like or as a syrup or elixir suitable for oral administration. For intravenous or intraperitoneal administration, the compound will be prepared as a solution or suspension capable of being administered by injection. In certain cases, it may be useful to formulate these compounds by injection. In certain other cases, it may be useful to formulate these compounds in suppository form or as extended release formulation for deposit under the skin or intramuscular injection.

The compound of **Formula 1** will be administered as a chemotherapeutic agent for treatment of tumors in a useful therapeutic dose which will vary from condition to condition and in certain instances may vary with the severity of the condition being treated and the patient's susceptibility to treatment. Accordingly, no single dose will be uniformly useful, but will require modification depending on the particularities of the tumor or malignancy being treated. Such doses can be arrived at through routine experimentation. For the treatment of solid tumors and leukemias, particularly breast cancer and acute myeloid leukemia, it is anticipated that the compound of **Formula 1** will be administered for approximately 1 to 8 weeks to a patient in need thereof, in a dose that is effective to halt, slow the growth or dissipate the tumor or halt leukemia cell proliferation. Preferably, the

compound is to be administered orally, in a daily dose which preferably will be in the range of a approximately 50 mg per day to 500 mg per day. Most preferably the compound used in the treatment will be tazarotene (**Formula 3**).

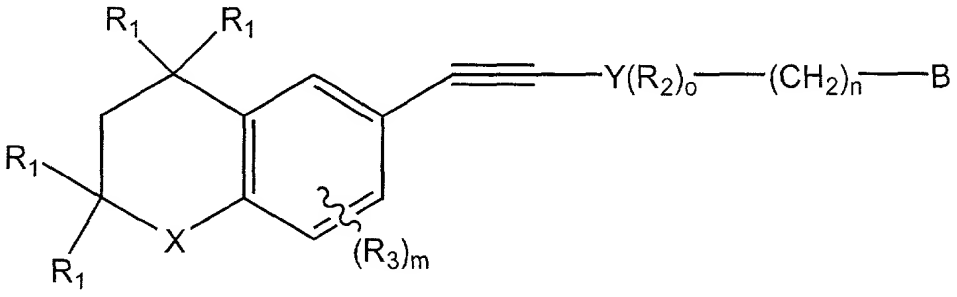
Preferably the compounds of **Formula 1**, and most preferably tazarotene (**Formula 3**) will be administered in combination with other chemotherapeutic agents, such as interferons, preferably human recombinant interferon, or other known chemotherapeutic agents of malignancies. Other chemotherapeutic agents with which the compounds of **Formula 1** are likely to be used in combination therapy are tamixofen and taxol. With the use of interferons and with certain other chemotherapeutic agents as well, a synergistic anti-proliferative, anti-tumor effect is likely to occur, as is demonstrated by the above described cell culture assay procedures. Again, when the compounds of **Formula 1** are used in a combination therapy, the useful therapeutic dose will vary from condition to condition and in certain instances may vary with the severity of the condition being treated and the patient's susceptibility to treatment. Accordingly, the required dose will be arrived at through routine experimentation, which is customary in the science of the chemotherapy of malignancies.

Generally speaking it is contemplated that in combination therapy and for the treatment of solid tumors and leukemias, the daily dose of the compound of **Formula 1** will be in the range of a approximately 50 mg per day to 500 mg per day. The daily dose of the other chemotherapeutic agent or agents given in combination with the compound of **Formula 1** will depend on the nature of the chemotherapeutic agent or agents, and can be arrived by routine experimentation normally practiced in the art. When interferon is used for the treatment of solid tumors or leukemias, such as for example breast cancer or acute myeloid leukemia, in combination with the compounds of **Formula 1**, then the daily dose of the interferon is likely to be in the range of

approximately 1 to 9 million international units per day.

WHAT IS CLAIMED IS:

1.. A pharmaceutical composition for the treatment of a malignant disease or condition in a mammal, the composition comprising a pharmaceutically acceptable excipient and a therapeutically effective dose of a compound of the formula



where $\mathbf{R_1}$ is independently H or lower alkyl of 1 to 6 carbons;

$\mathbf{R_2}$ and $\mathbf{R_3}$ are independently H, lower alkyl of 1 to 6 carbons, F, Cl, Br, I, alkoxy of 1 to 6 carbons, or fluoroalkoxy of 1 to 6 carbons;

m is an integer 0 to 3;

o is an integer 0 to 4;

n is 0-5;

Y is phenyl, naphthyl, or a heteroaryl group selected from a group consisting of pyridyl, thienyl, furyl, pyridazinyl, pyrimidinyl, pyrazinyl; oxazolyl, thiazolyl, or imidazolyl, and

B is COOH, a pharmaceutically acceptable salt thereof, $CONR_6R_7$ or $COOR_8$ where $\mathbf{R_6}$ and $\mathbf{R_7}$ independently are hydrogen or an alkyl group of 1 to 6 carbons and $\mathbf{R_8}$ is alkyl of 1 to 6 carbons,

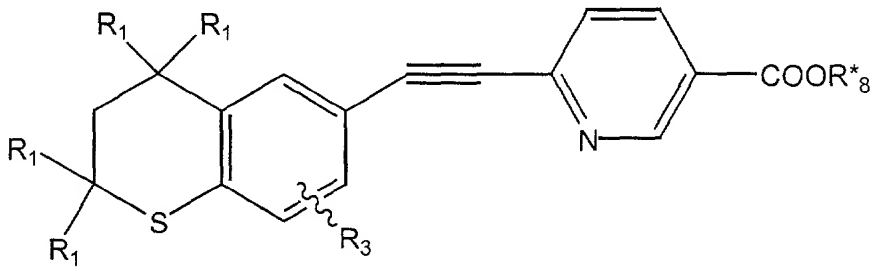
said composition being adapted to be used in combination with another chemotherapeutic agent effective for the treatment of the malignant disease or condition of the mammal.

2. A pharmaceutical composition in accordance with Claim 1 wherein the chemotherapeutic agent effective for the treatment of the malignant disease or condition of the mammal is interferon.

3. A pharmaceutical composition in accordance with Claim 2 adapted for the treatment of breast cancer.

4. A pharmaceutical composition in accordance with Claim 2 adapted for the treatment of leukemia.

5. A pharmaceutical composition in accordance with Claim 1 wherein the compound has the formula



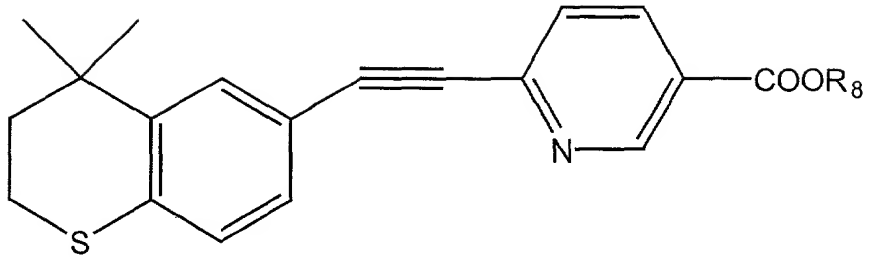
where $\mathbf{R_1}$ is H or methyl, $\mathbf{R_3}$ is H or methyl, and $\mathbf{R^*_8}$ is H, or lower alkyl of 1 to 3 carbons, or a pharmaceutically acceptable salt of said compound.

6. A pharmaceutical composition in accordance with Claim 5 wherein the chemotherapeutic agent effective for the treatment of the malignant disease or condition of the mammal is interferon.

7. A pharmaceutical composition in accordance with Claim 6 adapted for the treatment of breast cancer.

8. A pharmaceutical composition in accordance with Claim 5 adapted for the treatment of leukemia.

9. A pharmaceutical composition in accordance with Claim 1 wherein the compound has the formula



where $\mathbf{R_8}$ is H, alkyl of 1 to 3 carbons, or a pharmaceutically acceptable salt of said compound.

10. A pharmaceutical composition in accordance with Claim 9 wherein the chemotherapeutic agent effective for the treatment of the malignant disease or condition of the mammal is interferon.

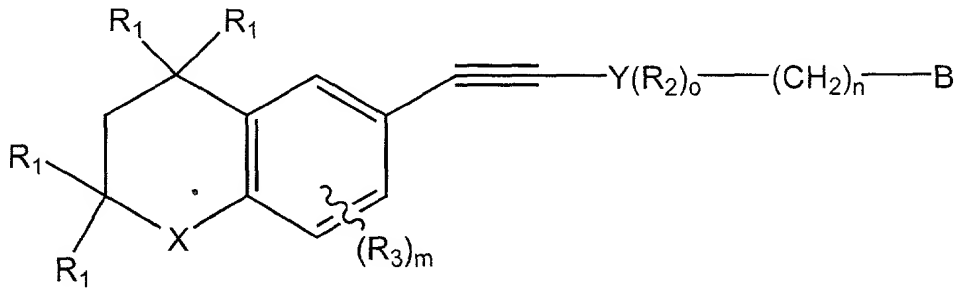
11. A pharmaceutical composition in accordance with Claim 10 adapted for the treatment of breast cancer.

12. A pharmaceutical composition in accordance with Claim 10 adapted for the treatment of leukemia.

13. A pharmaceutical composition in accordance with Claim 9 where $\mathbf{R_8}$ is ethyl.

14. A method of treating a malignant disease or condition in a mammal in need of such treatment, the method comprising the steps of:

administering to said mammal a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a therapeutically effective dose of a compound of the formula



where $\mathbf{R_1}$ is independently H or lower alkyl of 1 to 6 carbons;

$\mathbf{R_2}$ and $\mathbf{R_3}$ are independently H, lower alkyl of 1 to 6 carbons, F, Cl, Br, I, alkoxy of 1 to 6 carbons, or fluoroalkoxy of 1 to 6 carbons;

m is an integer 0 to 3;

o is an integer 0 to 4;

n is 0-5;

Y is phenyl, naphthyl, or a heteroaryl group selected from a group consisting of pyridyl, thienyl, furyl, pyridazinyl, pyrimidinyl, pyrazinyl; oxazolyl, thiazolyl, or imidazolyl;

B is COOH, a pharmaceutically acceptable salt thereof, $CONR_6R_7$ or $COOR_8$ where $\mathbf{R_6}$ and $\mathbf{R_7}$ independently are hydrogen or an alkyl group of 1 to 6 carbons and $\mathbf{R_8}$ is alkyl of 1 to 6 carbons, and

co-administering to said mammal with said compound another chemotherapeutic agent effective for the treatment of the malignant disease or condition of the mammal.

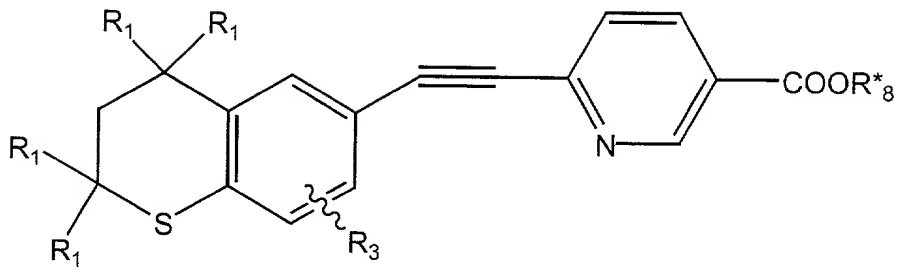
15. A method in accordance with Claim 14 where the chemotherapeutic agent is interferon.

16. A method in accordance with Claim 15 where the chemotherapeutic agent is human recombinant interferon α, human recombinant interferon β, or human recombinant interferon γ.

17. A method in accordance with Claim 16 where the malignant disease or condition treated is breast cancer or leukemia.

18. A method in accordance with Claim 17 where the malignant disease or condition treated is acute myeloid leukemia.

19. A method in accordance with Claim 14 wherein the compound has the formula



where $\mathbf{R_1}$ is H or methyl, $\mathbf{R_3}$ is H or methyl, and $\mathbf{R^*_8}$ is H, or lower alkyl of 1 to 3 carbons, or a pharmaceutically acceptable salt of said compound.

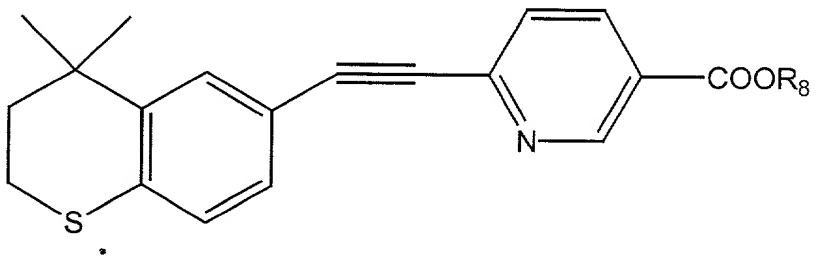
20. A method in accordance with Claim 19 where the chemotherapeutic agent is interferon.

21. A method in accordance with Claim 20 where the chemotherapeutic agent is human recombinant interferon α, human recombinant interferon β, or human recombinant interferon γ.

22. A method in accordance with Claim 21 where the malignant disease or condition treated is breast cancer or leukemia.

23. A method in accordance with Claim 21 where the malignant disease or condition treated is acute myeloid leukemia.

24. A method in accordance with Claim 14 wherein the compound has the formula



where $\mathbf{R_8}$ is H, alkyl of 1 to 3 carbons, or a pharmaceutically acceptable salt of said compound.

25. A method in accordance with Claim 24 where $\mathbf{R_8}$ is ethyl.

26. A method in accordance with Claim 25 where the chemotherapeutic agent is interferon.

27. A method in accordance with Claim 26 where the chemotherapeutic agent is human recombinant interferon α, human recombinant interferon β, or human recombinant interferon γ.

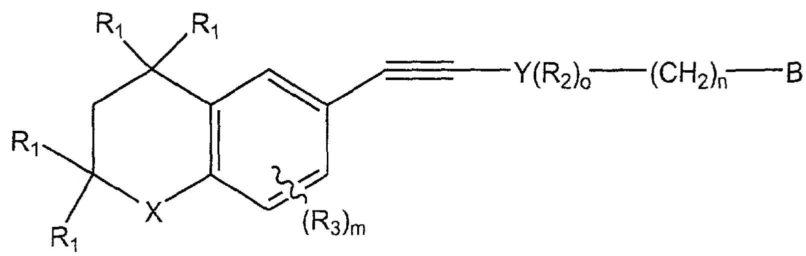
28. A method in accordance with Claim 27 where the malignant disease or condition treated is breast cancer or leukemia.

29. A method in accordance with Claim 27 where the malignant disease or condition treated is acute myeloid leukemia.

30. A method in accordance with any of the Claims 24 through 29 wherein a daily dose of approximately 50 mg to 500 mg of the compound is administered to the mammal.

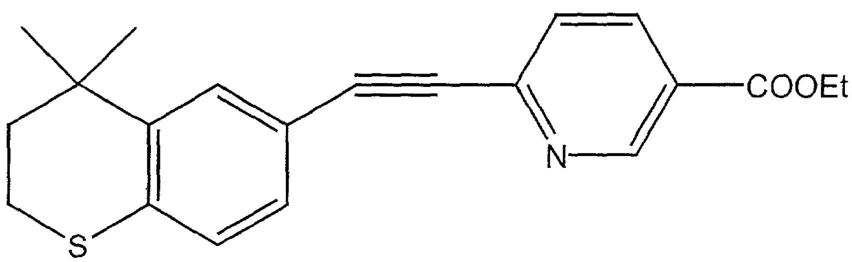
ABSTRACT OF THE DISCLOSURE

Compounds of **Formula 1**



FORMULA 1

where the symbols have the meaning described in the specification, and particularly the compound of **Formula 3**



FORMULA 3 (tazarotene)

exhibit synergistic anti-proliferative effect with human recombinant interferon in the treatment of solid tumors and leukemia, particularly breast cancer and acute myeloid leukemia.

Figure 1: cotreatment tazarotene at variable concentrations + IFN-a (100U/ml) in SK-BR-3 cells

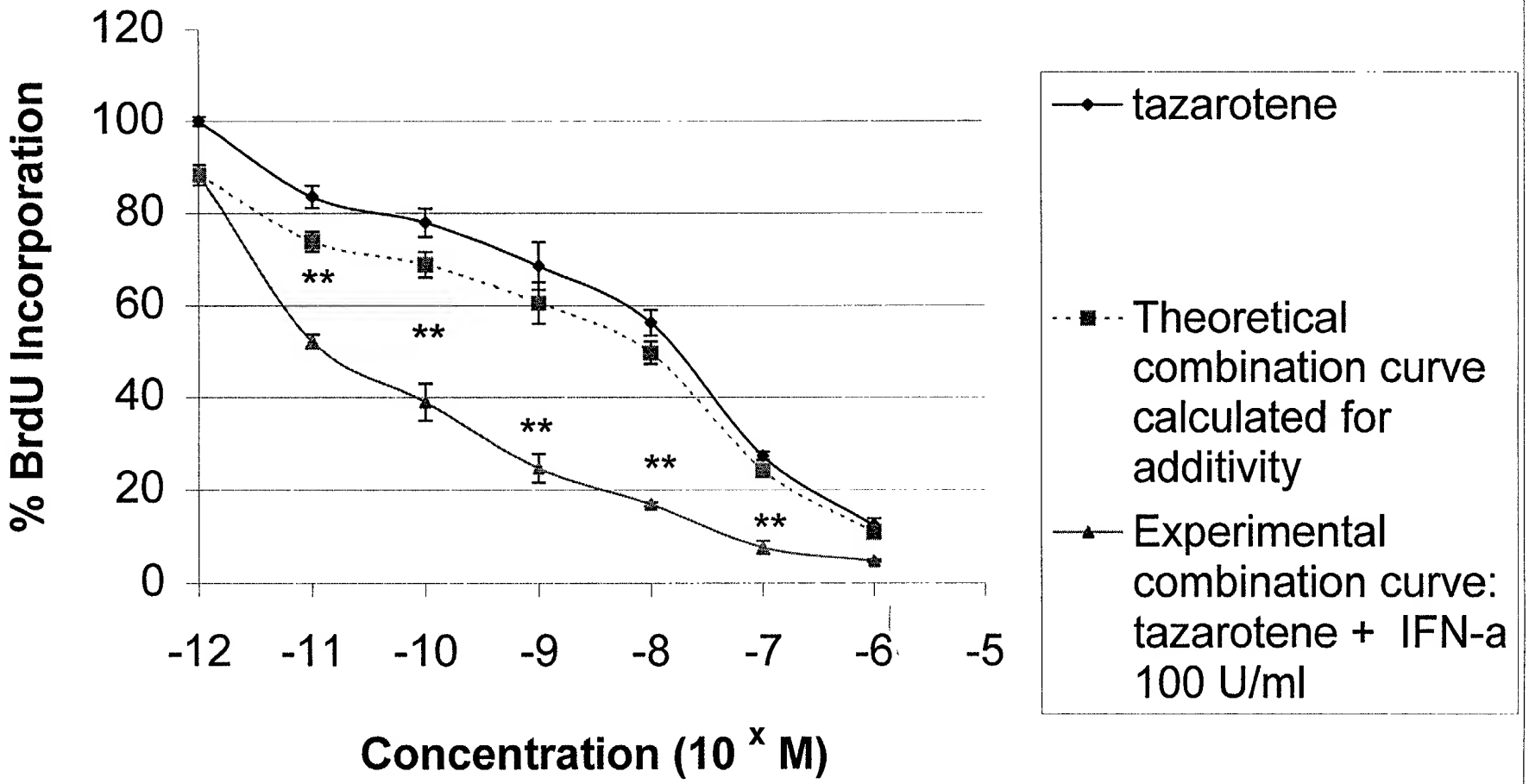


Figure 2: cotreatment tazarotene at variable concentrations + IFN-a (100U/ml) in T-47D cells

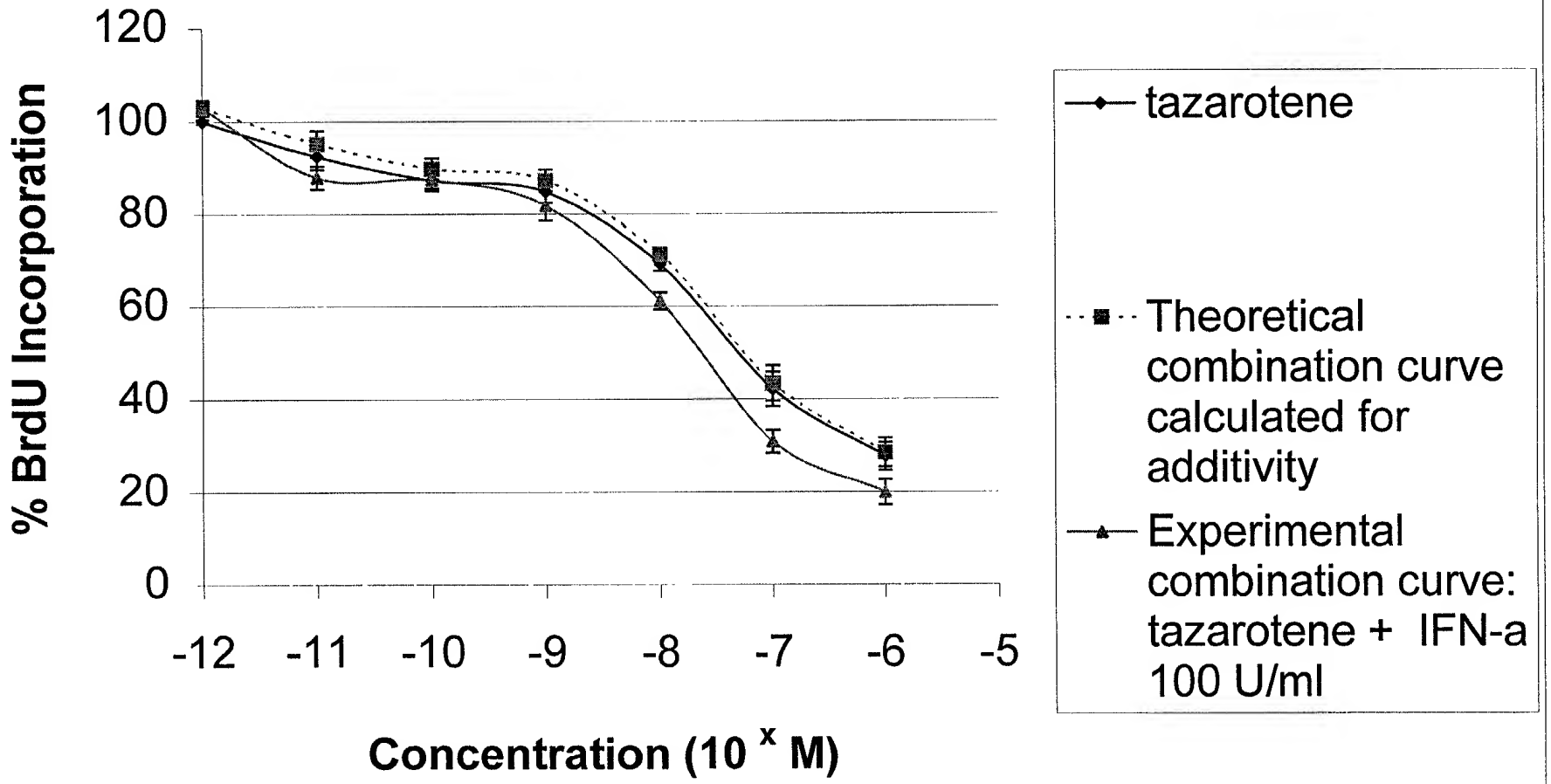


Figure 3: cotreatment tazarotene at variable concentrations + IFN-b (10 U/ml) in SK-BR-3 cells

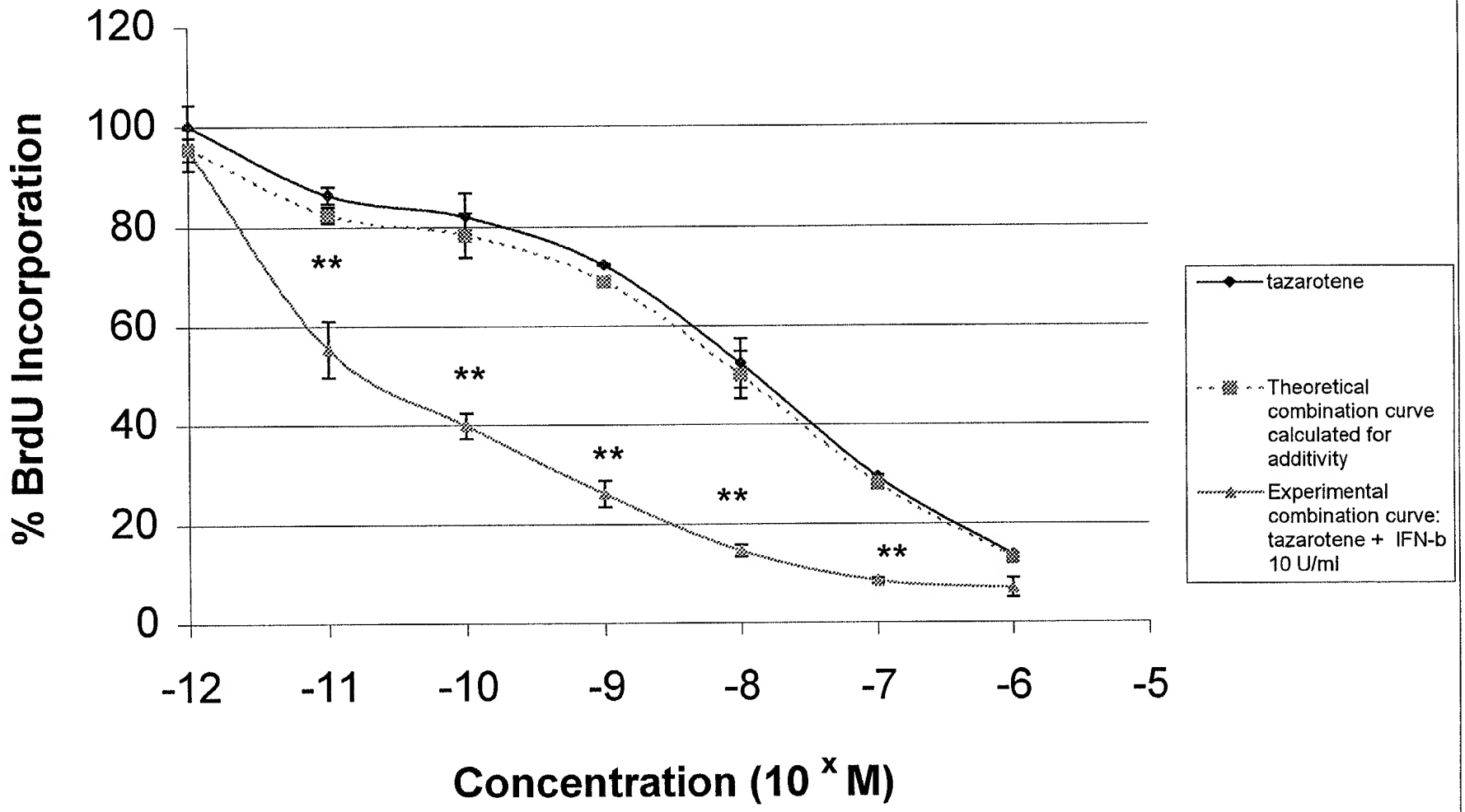


Figure 4: cotreatment tazarotene at variable concentrations + IFN-b (100 U/ml) in T-47D cells

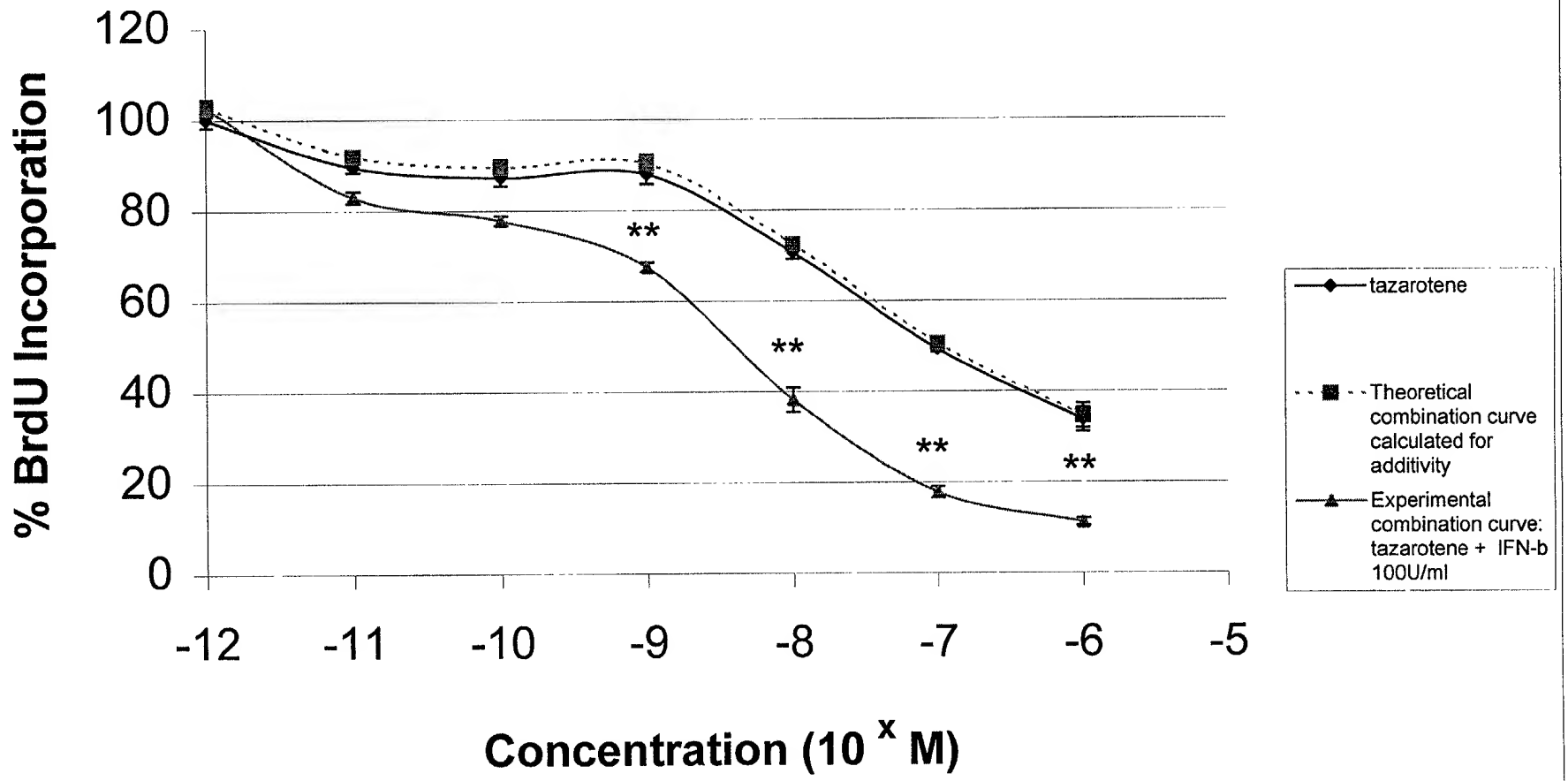


Figure 5: cotreatment tazarotene at variable concentrations + IFN-g (100 U/ml) in SK-BR-3 cells

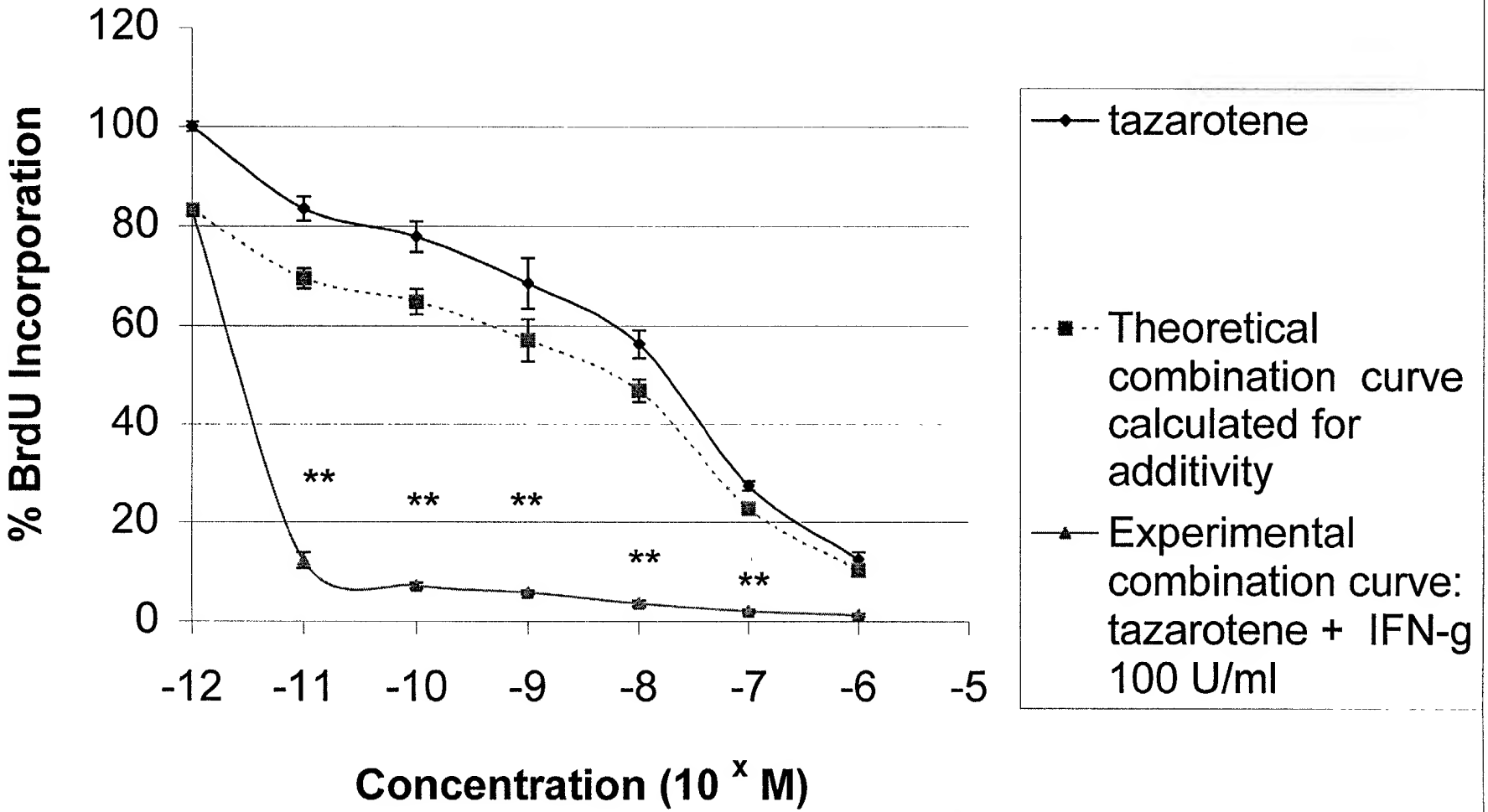


Figure 6: cotreatment tazarotene at variable concentrations + IFN-g (100 U/ml) in T-47D cells

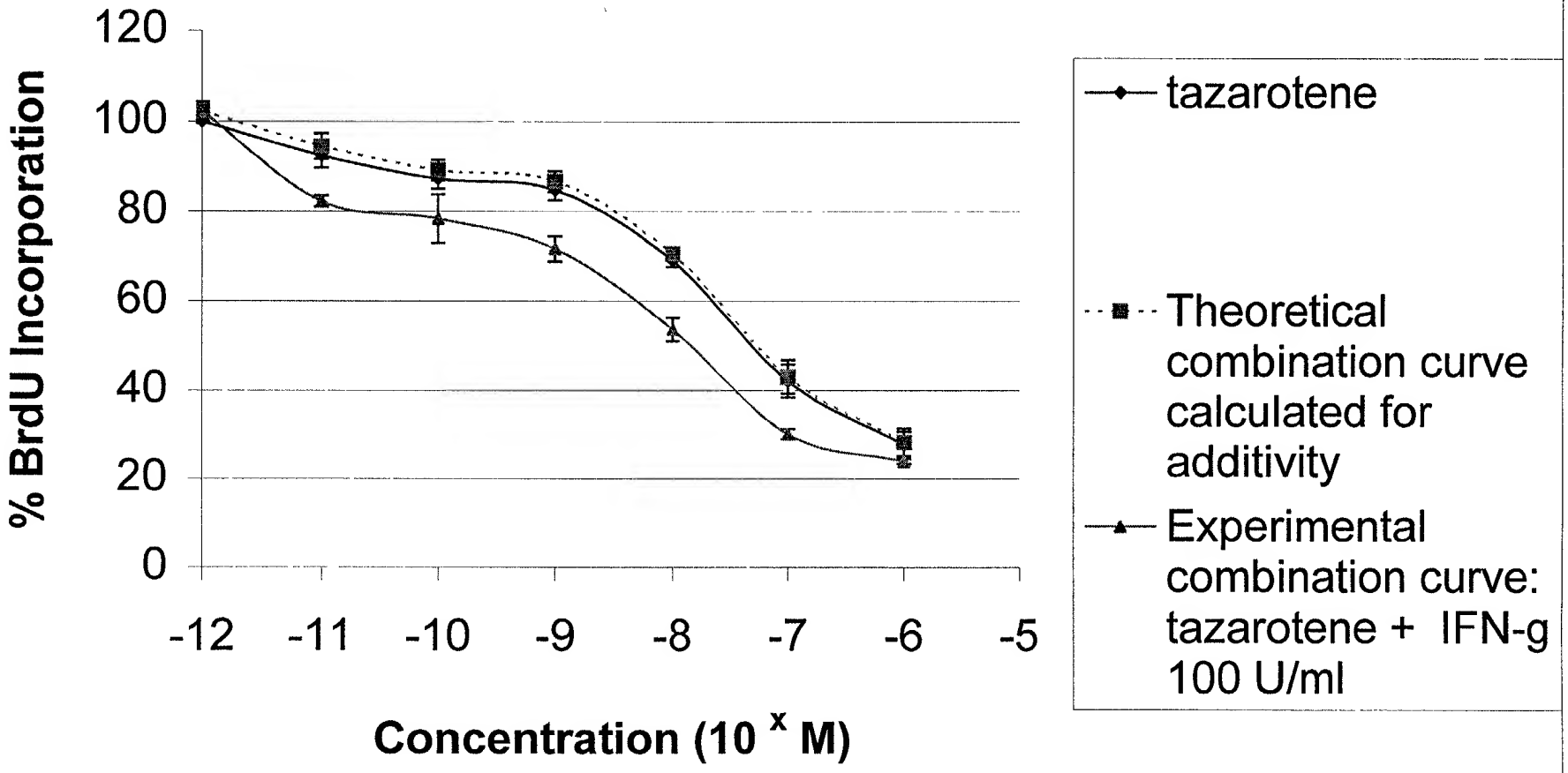


Figure 7: cotreatment IFN-a at variable concentrations + tazarotene (10 nM) in SK-BR-3 cells

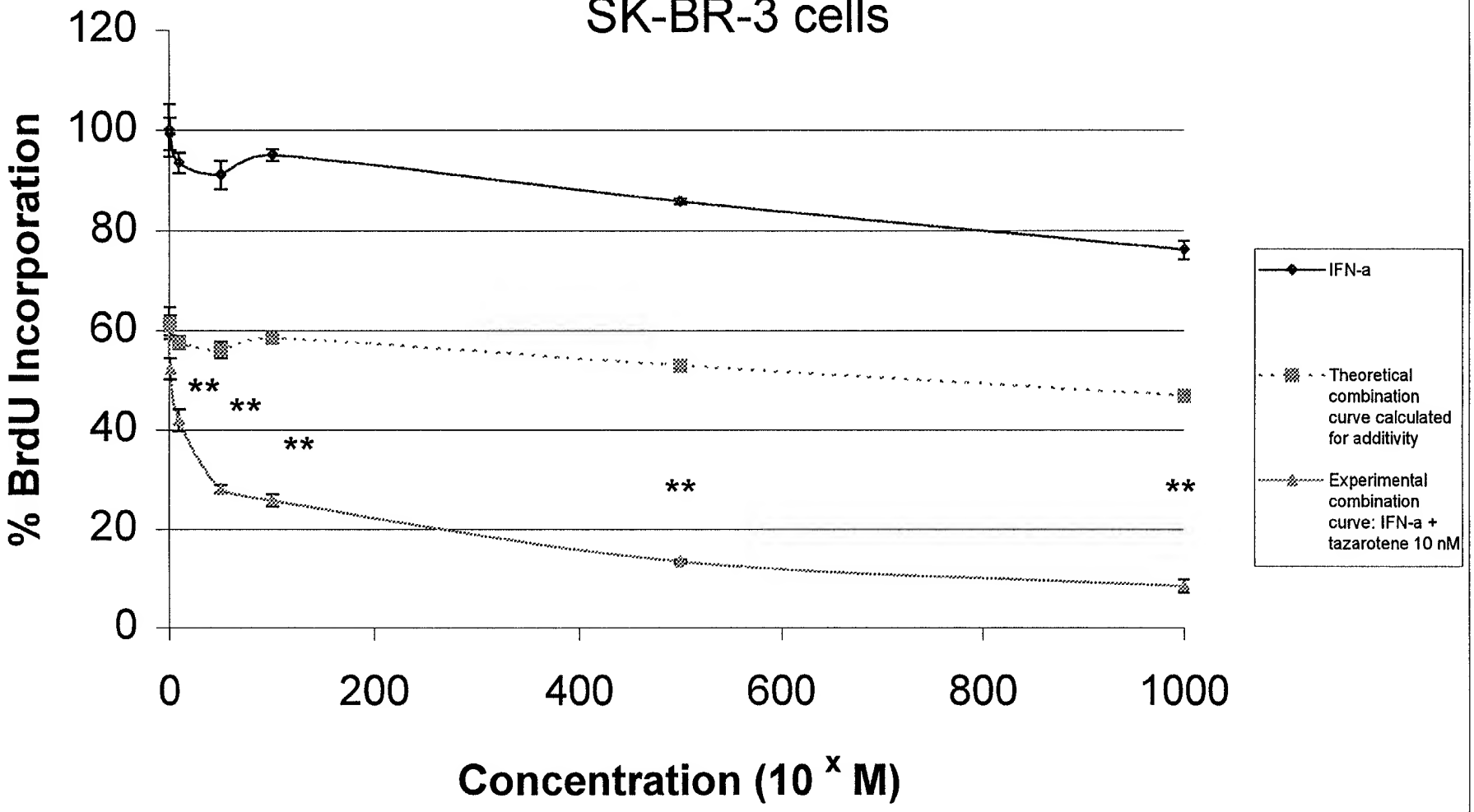


Figure 8: cotreatment IFN-a at variable concentrations + tazarotene (10 nM) in T-47D cells

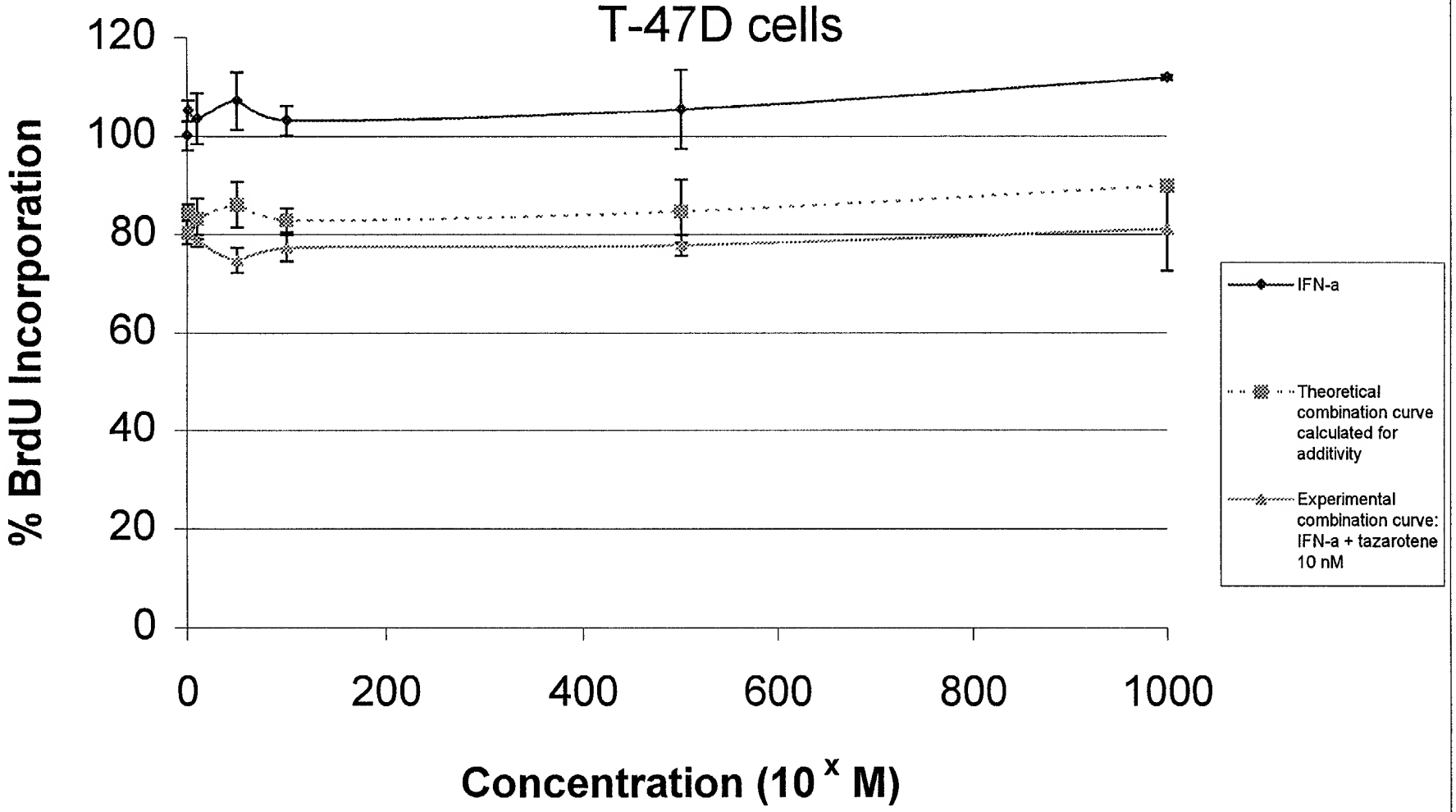


Figure 9: cotreatment IFN-b at variable concentrations + tazarotene (10 nM) in SK-BR-3 cells

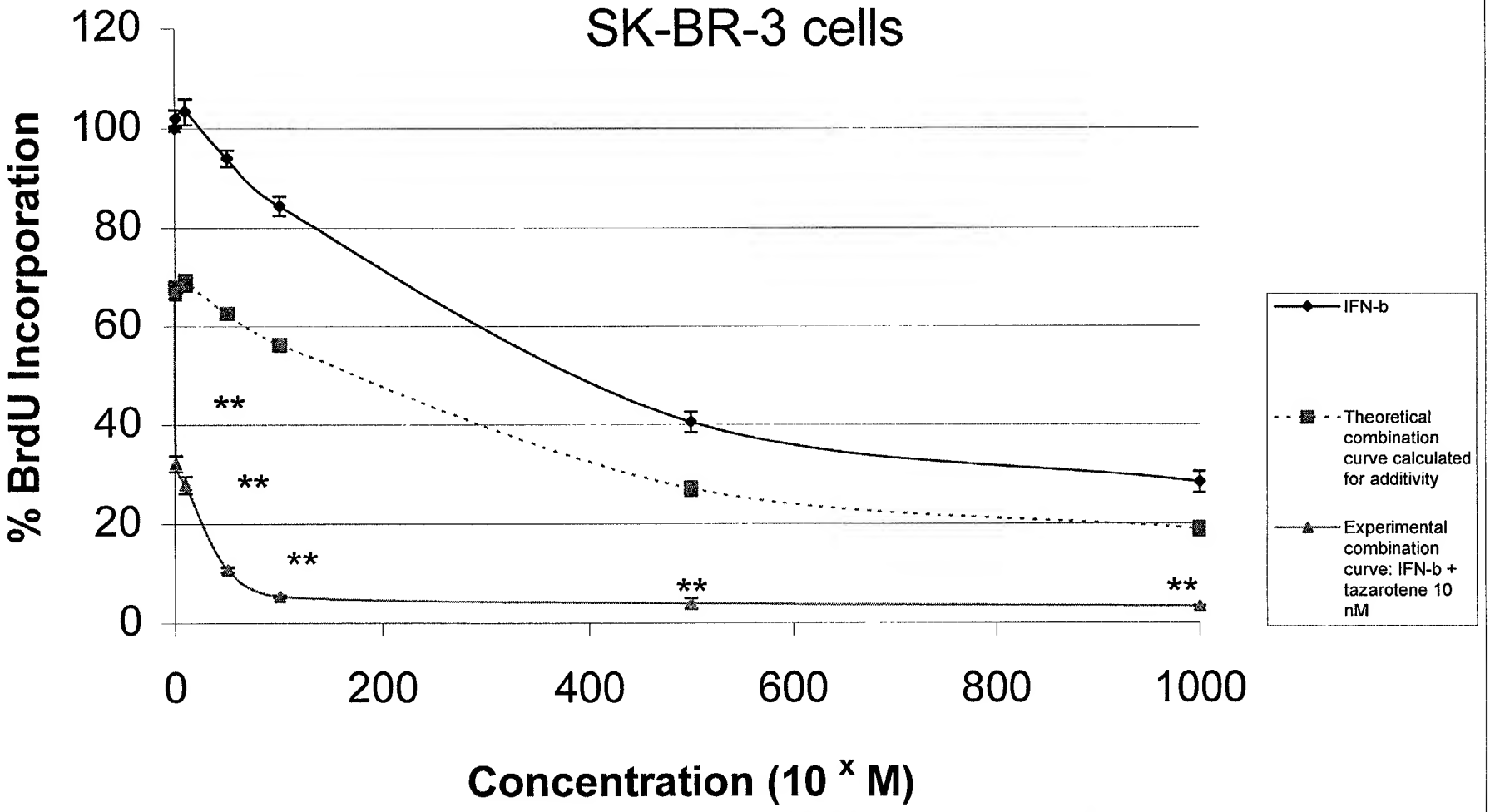


Figure 10: cotreatment IFN-b at variable concentrations + tazarotene (10 nM) in T-47D cells

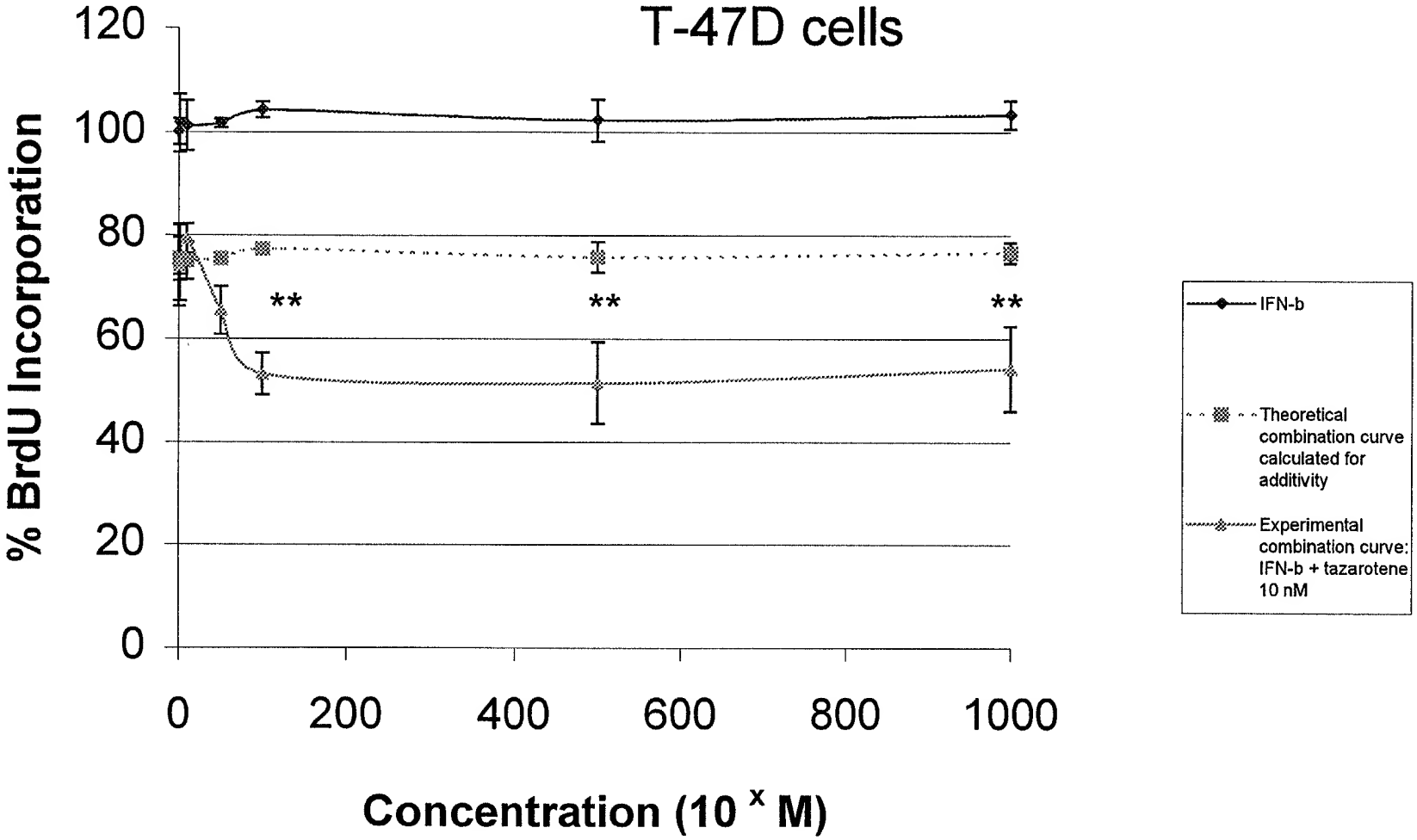


Figure 11: cotreatment IFN-g at variable concentrations + tazarotene (10 nM) in SK-BR-3 cells

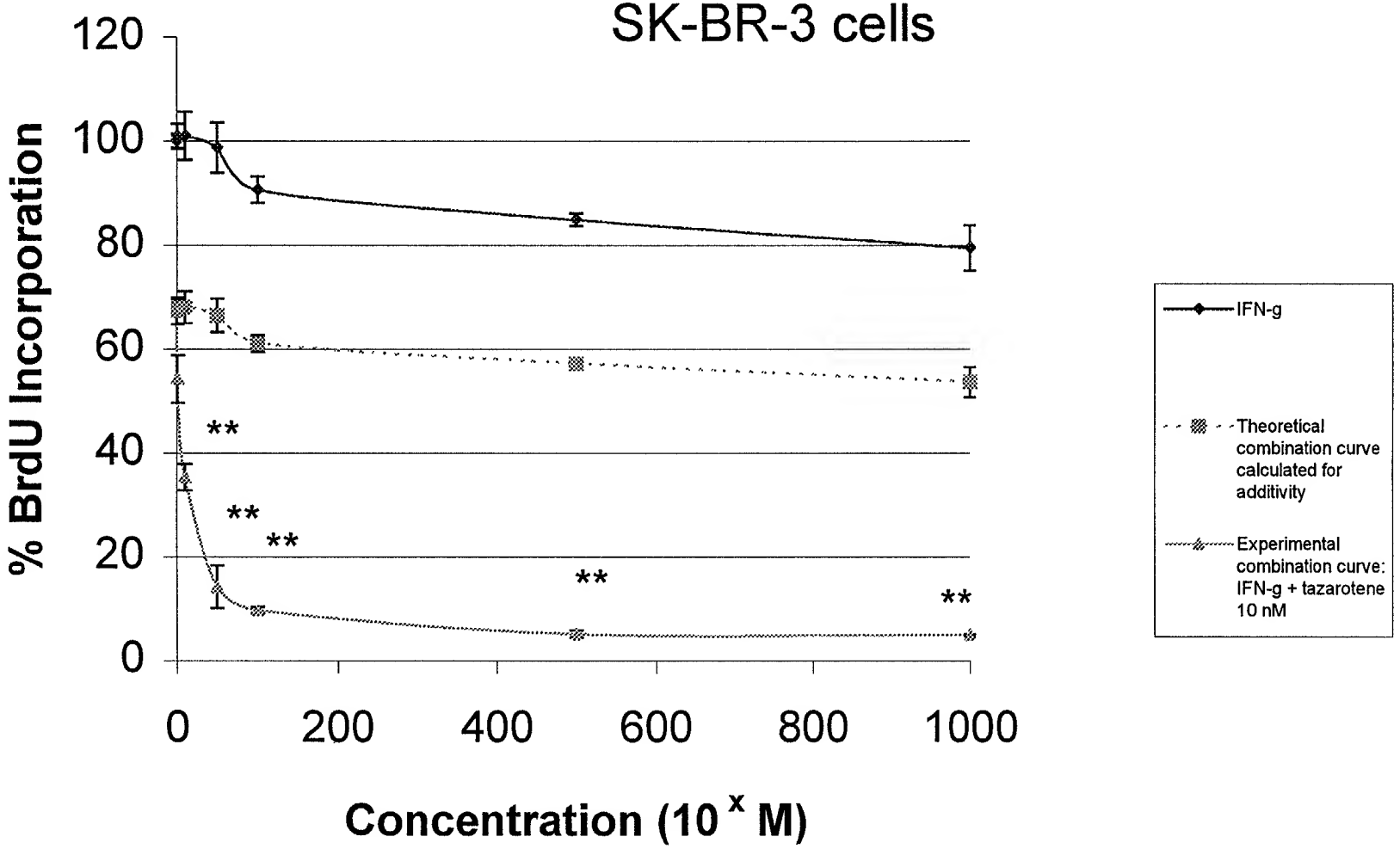


Figure 12: cotreatment IFN-g at variable concentrations + tazarotene (10 nM) in T-47D cells

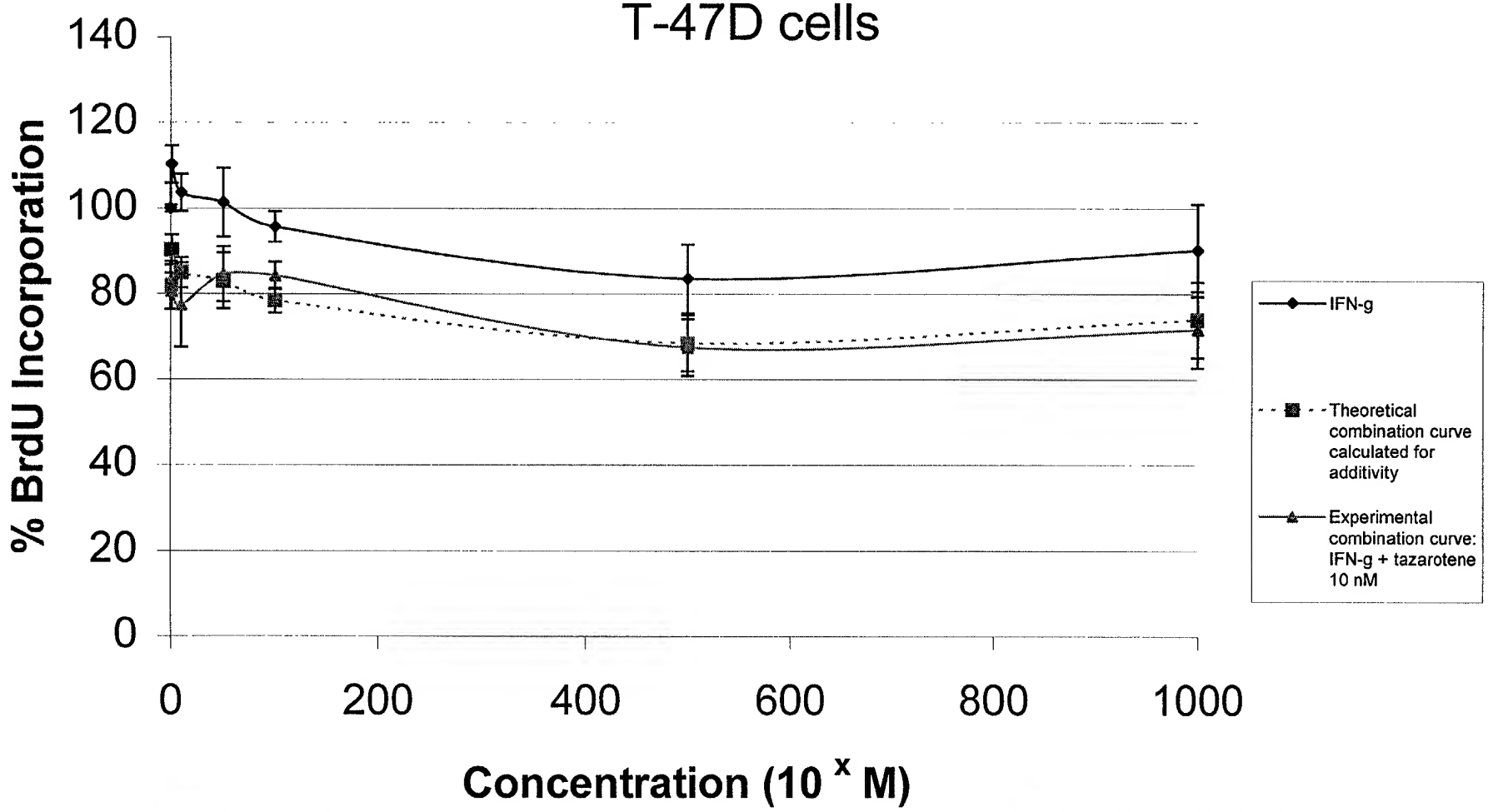


Figure 13: Cotreatment tazarotene at variable concentrations + IFN-alpha (100U/ml) in HL-60

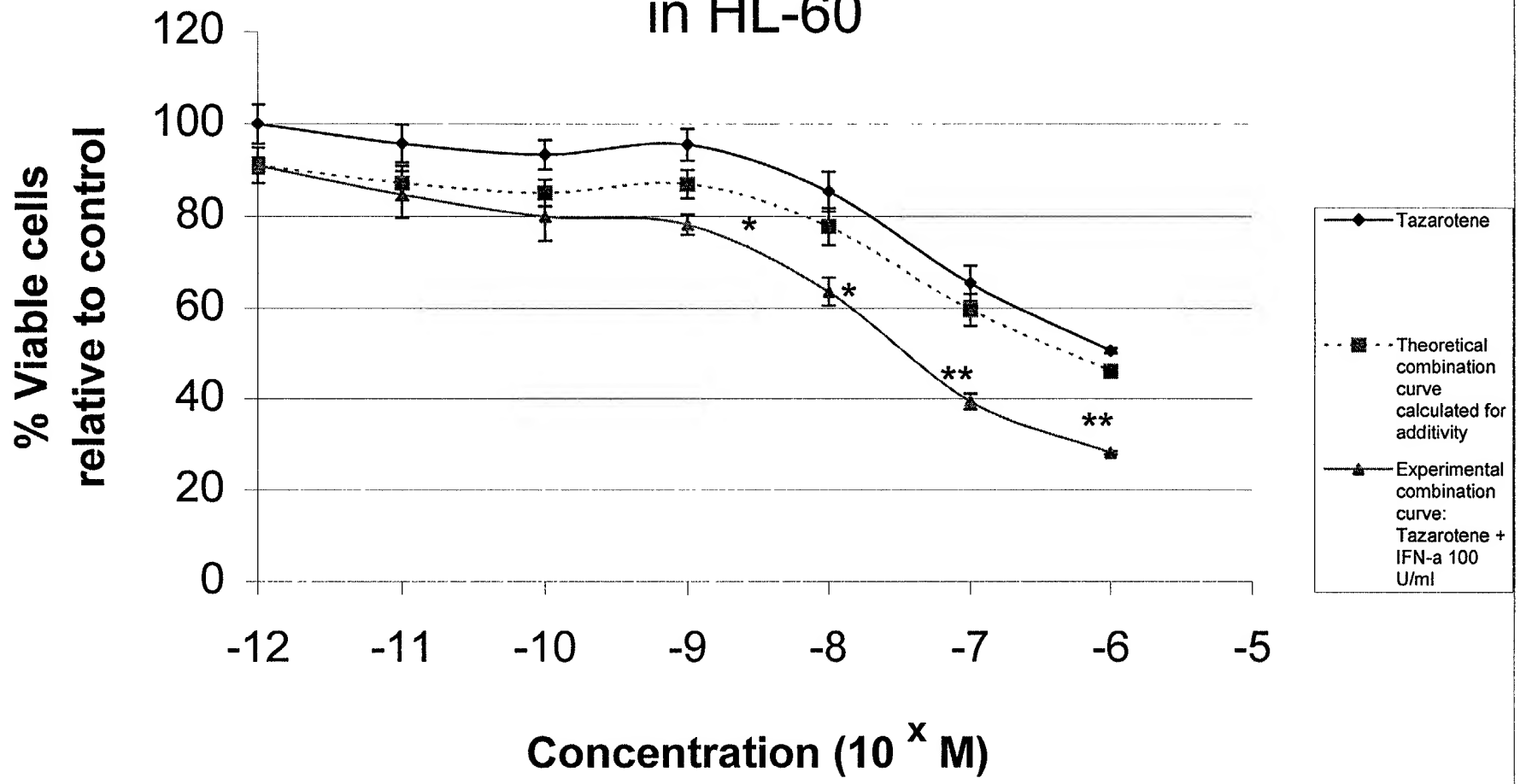


Figure 14: Cotreatment tazarotene at variable concentrations + IFN-beta (100U/ml) in HL-60

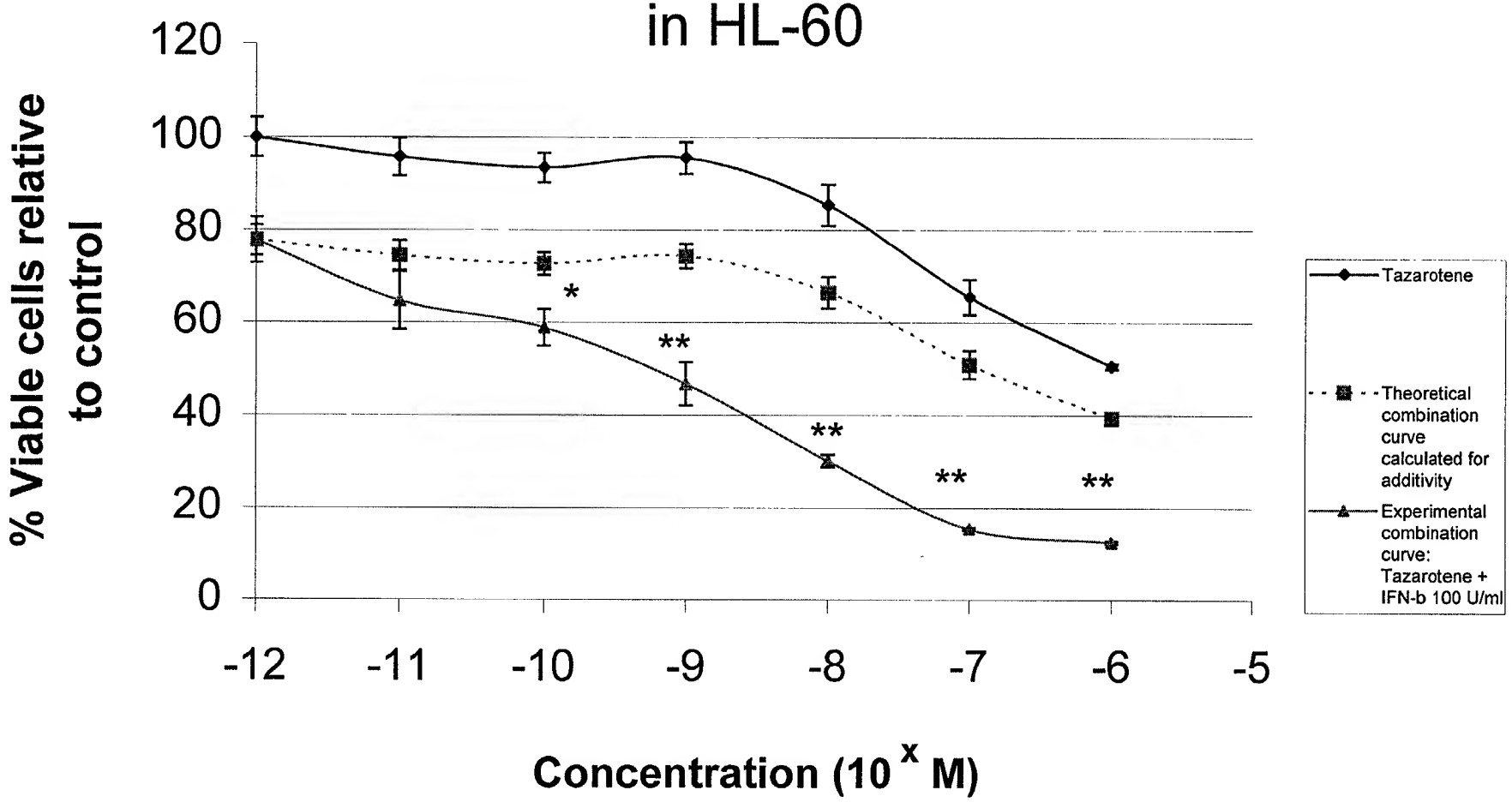


Figure 15: Cotreatment IFN-alpha at variable concentrations + tazarotene (50nM) in HL-60

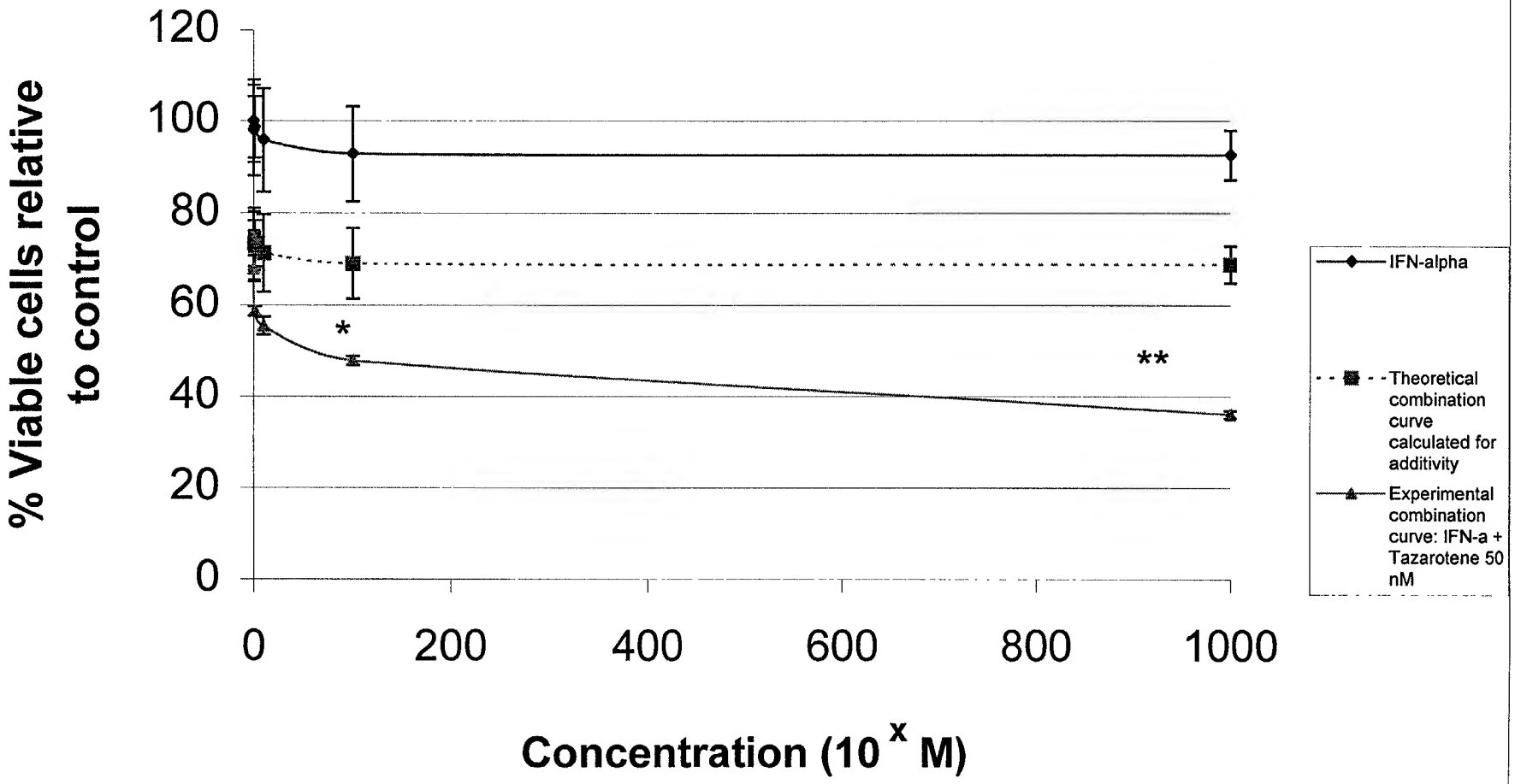
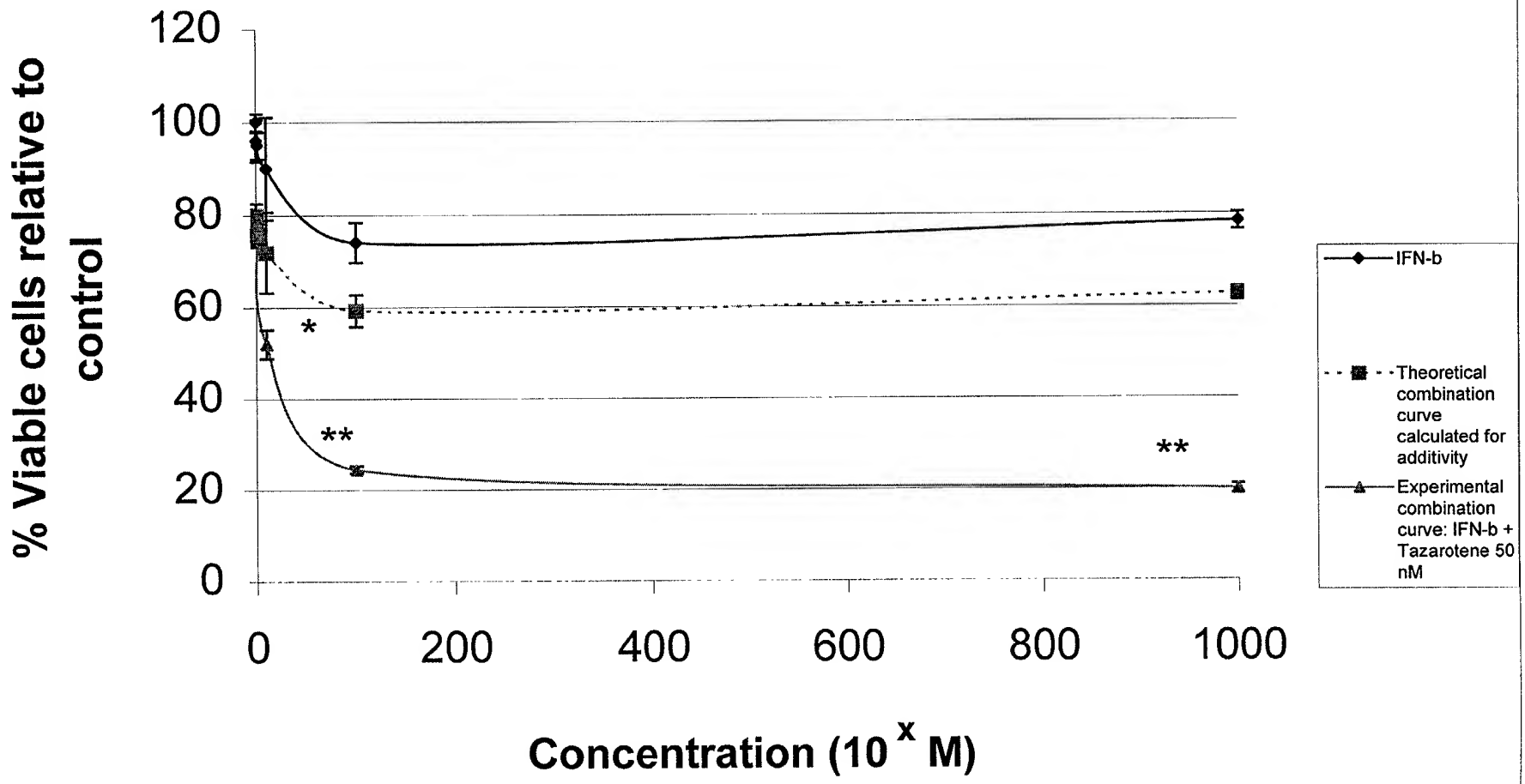


Figure 16: Cotreatment IFN-beta at variable concentrations + tazarotene (50nM) in HL-60



Atty. Docket No.
600-41-PA

PATENT

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below-named inventor, I HEREBY DECLARE THAT:

This Declaration is for the following type of application:

ORIGINAL

My residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor or the subject matter which is claimed and for which a patent is sought on the invention entitled **TREATMENT OF TUMORS WITH ACETYLENES DISUBSTITUTED WITH A PHENYL OR HETEROAROMATIC GROUP AND A SUBSTITUTED CHROMANYL, THIOCHROMANYL OR TETRAHYDROQUINOLINYL GROUP IN COMBINATION WITH OTHER ANTI-TUMOR AGENTS** the specification of which is attached hereto unless the following box is checked:

was filed on _____ as United States Application Number or PCT International Application Number_____ and was amended on_____(if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability of this application as defined in **37 CFR § 1.56**.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(Application Number)	(Filing Date)	(Status -- patented, pending, abandoned)
(Application Number)	(Filing Date)	(Status -- patented, pending, abandoned)

POWER OF ATTORNEY

I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Martin A. Voet	**Registration No. 25,208**
Robert J. Baran	**Registration No. 25,806**
Carlos Fisher	**Registration No. 36,510**
Stephen Donovan	**Registration No. 33,433**
Gabor L. Szekeres	**Registration No. 28,675**
Howard J. Klein	**Registration No. 28,727**

Please send all correspondence to:

KLEIN & SZEKERES, LLP
4199 Campus Drive, Suite 700
Irvine, California 92612

and address all Telephone Calls to:

Gabor L. Szekeres
(949) 854-5502

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of First Joint Inventor
Alissar NEHME

Citizenship
Lebanon

RESIDENCE Address - Street
1300 Park Newport, No. 416

POST OFFICE Address - Street
1300 Park Newport, No. 416

City (Zip)
Newport Beach 92660

City (Zip)
Newport Beach 92660

State or Country
California, U.S.A.

State of Country
California, U.S.A.

Date: 08/11/00

Signature

Full Name of Second Joint Inventor
Richard L. BEARD

Citizenship
U.S.A.

RESIDENCE Address - Street
2341 Azure Avenue

POST OFFICE Address - Street
2341 Azure Avenue

City (Zip)
Newport Beach 92660

City (Zip)
Newport Beach 92660

State or Country
California U.S.A.

State of Country
California U.S.A.

Date: 8/11/2000

Signature

Full Name of Third Joint Inventor	Citizenship
Roshantha A. Chandraratna	**U.S.A.**
RESIDENCE Address - Street	POST OFFICE Address - Street
25241 Buckskin	**25241 Buckskin**
City (Zip)	City (Zip)
Laguna Hills 92653	**Laguna Hills 92653**
State or Country	State of Country
California U.S.A.	**California U.S.A.**

Date: 8/11/2000 R. A. Chandraratna
Signature